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SPACE BIOLOGY AND AEROSPACE MEDICINE

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CIRCULATORY ORTHOSTATIC INSTABILITY: ROLE OF DECONDITIONING OF RESISTIVE VESSELS

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 18, No 4, Jul-Aug 84 (manuscript received 9 Feb 83) pp 4-12

[Article by V. M. Khayutin, S. M. Shenderov, A. G. Zakharov and A. N. Rogoza]

[English abstract from source] One of the effects of weightlessness on the circulatory system, i.e., reduction of the tension distending resistance vessels due to the loss of hydrostatic pressure, was simulated. For this purpose the abdominal aorta of rats was constricted by a wire spiral. As a result, the arterial pressure in the posterior body decreased by 30-50%. Beginning with postoperation day 7 the hydraulic resistance of resistance vessels of hindlimbs was decreased progressively and the myogenic regulation of their lumen was inhibited. The response to the stimulation of vasoconstrictive fibers of the sciatic nerve that was reduced 7-14 days after operation returned to normal 90 days later. An examination of the tail arteries demonstrated that the ability of their muscular layer to withstand the distending effect of the physiologically normal pressure was impaired. The magnitude of the constrictor reaction to norepinephrine (particularly to its moderate concentrations) was lowered. These changes may play an important role in the orthostatic disorders of circulation that occur after exposure to weightlessness.

[Text] When we are standing or sitting, hydrostatic forces generate additional distending tension in the walls of resistive vessels of the legs, which should lead to enlargement of their lumen and increased blood flow. However, this does not usually occur: in orthostatic position, there is intensification of both discharges of vasoconstrictor fibers directed to muscle vessles [14] and, probably, of the Bayliss myogenic resp-nse [18], so that resistive vessels can counteract hydrostatic pressure. For such counteraction to be effective, the vessels as a whole, as a morphofunctional structure, must have some "normal" properties which, like the properties of any tissues, are maintained by mecha isms of long-term cellular regulation, i.e., they are the result of some sort of conditioning factors.

It is logical to assume that intravascular pressure and its elevation in orthostatic position, which periodically increases the load on vascular walls, serves as the conditioning factor that provides for "normal" properties of vessels.

Weightlessness creates different conditions: since there are no hydrostatic forces, there is attenuation of load on vessels. If there is validity to the view that the hydrostatic component of pressure is included in the vessel-conditioning stimulus, absence of this component in weightlessness could lead to a change in functional properties of resistive vessels--attenuation of both the myogenic reaction and reactions to discharges of constrictor neurons--and even to structural change in the vessels. When there is restoration of the effect of hydrostatic pressure on such resistive vessels, which have been deconditioned by its prolonged absence, they will present less resistance to displacement of blood in the venous system, which could serve as one of the causes of development of orthostatic instability. To prove the validity of this hypothesis [2], it is necessary to determine whether indeed prolonged pressure drop in resistive vessels leads to the expected changes in their properties.

Long-term local decline of pressure in arteries of animals could serve as a simple procedure for demonstration and analysis of the assumed deconditioning of vessels. To produce it, the aorta of white rats was constricted below the renal arteries (under sterile conditions) using coiled wire. Its diameter was so selected as to lower pressure in the femoral artery by 30-50%, as compared to pressure in the carotid. At different intervals thereafter (3 to 90 days after this operation), studies were made of mechanical properties and regulatory reactions of vessels of the hind legs, as well as caudal artery, in acute experiments [8, 4, 20].

The objection could be raised that such a procedure is not adequate to the problem: the pressure drop immediately decreases delivery of blood to the limbs and all subsequent changes in vascular properties could be due to this. However, measurements revealed that, already after 3 days and throughout the subsequent experimental period, blood supply to hypotensive limbs did not differ from normal, although the pressure in the femoral artery remained stably 30-50% lower [2, 4].

If we consider the fact that no collateral arteries develop in the hypotensive extremities [5] and that there is no increase in number of patent capillaries [3], normalization of blood flow should be due to increased hydraulic conduction of expressly resistive vessels. In order to find the cause of such increase, it is necessary to determine how the vessels of the extremities react to elevation of pressure in them. For this purpose, one of the hind legs was denervated and separated from the trunk high on the thigh 7, 14, 30 or 90 days after producing stenosis of the aorta. The extremity connected to the rat's body only by the femur and femoral vein was perfused with the animal's own blood, pumping it from the carotid into the femoral artery. A pump was used for this purpose, which was controlled by a servosystem that made it possible to set and maintain the required pressure in the femoral artery. The temperature of the animal's body and blood at the entrance to the limb was about 37°C.

A record of one of the experiments (Figure 1a) shows that, with elevation of pressure, there is rapid increase in blood flow followed by some decrease. The decrease is a manifestation of myogenic control of resistance of resistive vessels. Figure 1b illustrates the established values for blood flow as a function of perfusion pressure. As can be seen in Figure 1, the longer the hypotension, the greater the blood flow at each given pressure. Figure 1c

shows that, with increase in duration of hypotension, there is increasing decline of hydraulic resistance of resistive vessels over the entire range of pressures, i.e., their overall lumen grows progressively.

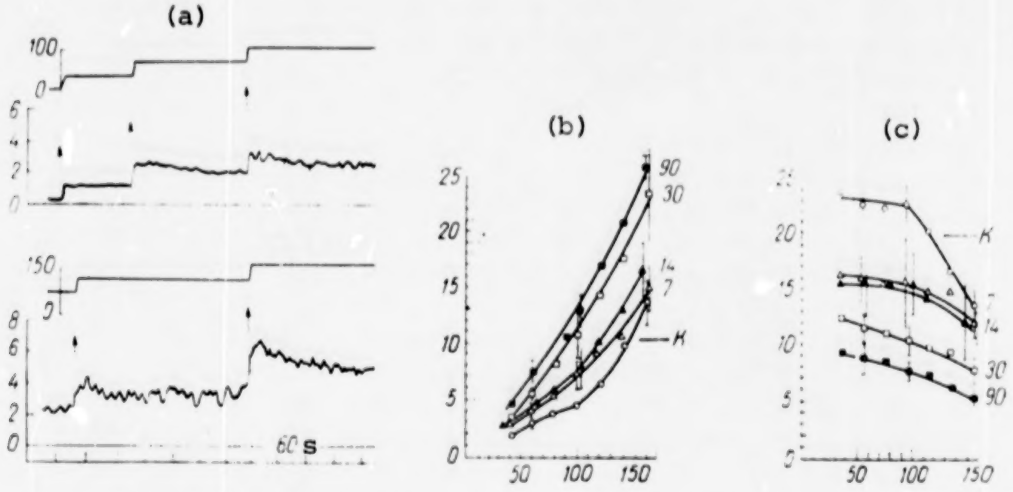

Figure 1. Blood flow in rat's hind leg (a--single experiment, b--mean data) and hydraulic resistance of its resistive vessels (c) as a function of perfusion pressure

a) top--perfusion pressure (mm Hg), bottom--blood flow (mℓ/min)
b and c) x-axis--perfusion pressure (mm Hg), y-axis--blood flow (mℓ/min); c--units of resistance
K) control
Numerals near curves show duration (in days) of hypotension.

At the same time, there is worsening of the capacity of resistive vessels to actively counteract distending pressure. In control rats, well-marked myogenic regulation of hydraulic resistance is observed in the range of 40-100 mm Hg. In this range, pressure increases by 2.5 times, while vascular resistance to blood flow, i.e., overall cross-section area of the arterial bed, undergoes virtually no change. At higher pressures, resistance to blood flow diminishes.* However, while myogenic regulation is still retained on the 7th-14th days of hypotension, it is lost by the 30th day. Consequently, the very existence of this important myogenic mechanism, which helps vessels to resist distension when loaded with intravascular pressure, is maintained by normal arterial pressure level [4, 20].

It is important to mention that maximum increase in hydraulic conduction occurs within the first 30 days after decline of pressure. If one restores normal pressure in the femoral artery (100 mm Hg) of rats submitted to local hypotension

*The narrow range of myogenic stabilization of hydraulic resistance in control rats is apparently due to the dissimilar intensity of the myogenic reaction of resistive vessels of the muscles, skin and bone tissue, as a result of which blood may be directed mainly to tissues, the vessels of which have inherently weaker myogenic regulation of their lumen.

for this period of time, influx of blood to the denervated extremity is 2.3 times greater than in control animals at the same pressure. When hypotension is extended to 90 days, absolute blood flow increases by only 20%. Thus, there is drastic slowing of increase in overall area of arterial cross-section after 30 days of hypotension. But, what causes this increase, attenuation and loss of the myogenic mechanism of stabilizing the vascular lumen alone, or this along with morphological changes in them?

In order to detect possible changes, the arterial system of the animals was filled with a dispersion of lead carbonate in gelatin solution under pressure of 100 mm Hg. Measurement was made of the thickness of the walls of small arteries and arterioles, as well as their inside diameter [5], on stained preparations of hindlimb muscles. The walls of all arterial vessels distal to the aortic stenosis were found to be thinner than in control animals. There was also a decrease in ratio of wall thickness to inside diameter, and this process progressed as the period of hypotension increased. This applies to all classes of resistive vessels that were examined, with lumen of 120 to 7 μm. There were no cytological data that would permit characterization of wall thinning as hypotrophy, but it can be noted that, after 30 days of hypotension or more, there was thinning of myocyte layers and decrease in volume of their nuclei in the wall of the aorta below the stenosis and in femoral arteries [5].

However, we cannot say what precisely the histological study demonstrates: actual decrease in mass of hypotensive vessel wall or sequelae of increased vascualr distensibility? If the vessels become more distensible, this should lead to decrease in thickness of the wall and enlargement of the lumen even with an unchanged cross-section. Hypotensive vessels were filled under pressure of 100 mm Hg in order to compare them to normotensive vessels. Of course, one could lower pressure when filling hypotensive vessels, but then this must also be done for control rats, i.e., create conditions that would alter the morphological "norm."

Consequently, in order to determine the causes of change in vascular resistance by distending forces, morphological data are needed and they should be affected by attenuation of vascular resistance to distention due to changes in their geometric dimensions and rigidity of materials of the vascular wall, not to mention attenuation of the myogenic response. For this reason, it is rather difficult to demonstrate involvement of morphological change in resistance vessels in increasing overall area of their lumen, but we shall return to the question of such change below in our discussion of changes in properties of small arteries.

In order to assess nervous regulation of resistive vessels, the effect on them of tonic discharges of vasoconstrictive fibers was removed, as illustrated in Figure 2a, by transecting the femoral and sciatic nerves, after which the latter's constrictor fibers were stimulated [4]. The changes that then occurred in blood flow were recorded, establishing 100 mm Hg perfusion pressure in the femoral artery. This pressure corresponds to the pressure in control rats and, what is very important, it permits determination of intensity of effect of discharges of constrictor fibers on hypotensive vessels when they are distended by expressly ordinary, "normal" pressure. In the model used, this corresponds to the condition of restoration of effect of hydrostatic forces on vessels that had been deconditioned by absence of these forces.

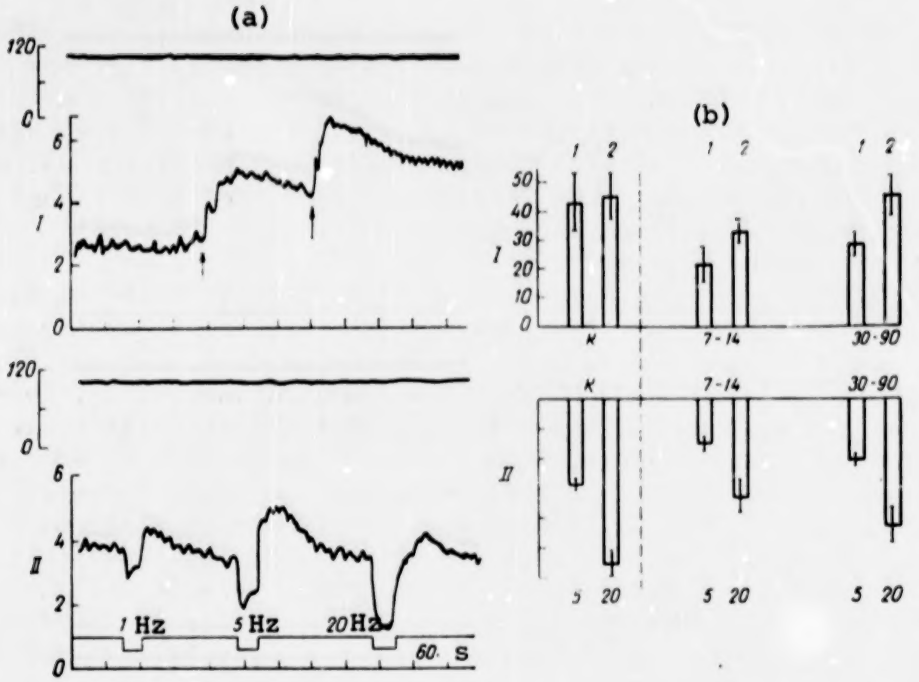

Figure 2. Changes in blood flow in rat's hind limb after transection of femoral and sciatic nerves (a,I and b,I) and stimulation of vasoconstrictor fibers of sciatic nerve (a,II and b,II)

a) individual experiment; a,I has arrowheads showing time of transection of femoral and sciatic nerves; a,II has numerals showing frequency (Hz) of nerve stimulation; a,I and II at the top--perfusion pressure (mm Hg), bottom--blood flow (mℓ/min)

b) mean data; y-axis--magnitude of reaction (%); numerals at base of bars--duration of hypotension (days); b,I: 1--transection of femoral nerve, 2--transection of sciatic nerve; b,II: bottom--frequency (Hz) of stimulation

K) control

Figure 2b illustrates mean data from such experiments. In control rats, the blood flow almost doubled after transection of both nerves. Consequently, tonic nervous control of resistive vessels of the hind legs is quite effective in this animal species.

Hypotensive vessels also react to transection of nerves by dilating. However, dilatation was substantially less marked on the 7th-14th days of hypotension.

According to Figure 2b, vessels submitted to low pressure for 7-14 days constrict upon stimulation of constrictor fibers about one-half less than normotensive vessels. Thus, the effectiveness of nervous control worsens appreciably in the first period of hypotension. It should be noted that drastic attenuation of constrictor reactions of chronically hypotensive vessels of posterior extremities occurs not only in rats, but also in cats [22] and dogs [13]. The results of theoretical analysis [6, 11] showed that, other conditions being equal, shortening of myocytes in response to a constrictive stimulus should be all the less marked, the thinner the vascular wall and the wider its lumen. Consequently,

if it is assumed that the walls of hypotensive vessels become thinner and their lumen wider, from the standpoint of mechanics it can be maintained that the rigidity of such vessels as a structure is weakened. Then it is easy to explain why there is worsening of their capacity to restrict blood flow when normal pressure is restored. Regardless of what causes thinning of the wall and enlargement of the vascular lumen (attenuation of myogenic stabilization of wall rigidity or morphological alteration), myocytes of hypotensive vessels must become shorter against the elevated pressure in their wall. For this reason, such vessels become less efficient blood flow regulators, particularly when they must reduce their lumen at normal, let alone elevated, pressure.

However, the reactions of resistive vessels to constrictor fiber impulses are particularly attenuated only at the first stage of chronic hypotension. With increase in its duration, there is a tendency toward restoration of effectiveness of tonic discharges, while the reactions to stimulation of constrictor fibers increase and reliably fail to differ from the reactions of normotensive vessels (see Figure 2b). Such a phenomenon cannot be attributed to diminished vascular rigidity as constriction, and one must resort to ad hoc hypotheses, for example, refer to the possibility of increased sensitivity of myocytes to an adrenergic transmitter or decrease in effective diffusion distance between sites of secretion of transmitter and myocytes, etc.

The mechanisms of changes that occur during chronic hypotension can be comprehended more fully if we refer to analysis of distensibility and constrictor reactions of a simpler effector, one of the small major arteries, for example, the rat's caudal artery [8]. Its muscular sheat constitutes 75% of the area of the cross section [19], so that this artery is a rather potent regulator of blood flow.

After ligating the lateral branches, a segment of the artery was placed in an incubation chamber filled with Tyrode's solution. Blood from the artery was continuously delivered into the femoral vein. A pneumatic cuff was used to ligate the segment of the artery at the base of the tail, while the distal end of the vessel was connected to a pressure-elevating device only for the duration of the study of arterial distensibility. Blood in the transfer tubes went into the artery and the latter stretched at each step of pressure elevation (steps of 20-40 mm Hg). The absolute diameter of the artery in each established state was measured optically, checking that a given state was reached by means of a capacitive sensor [7].

Figure 3a and 3b compares the increase in diameter of normotensive and hypotensive arteries as a function of distending pressure in two states: in one of them, the myocytes were activated by adding norepinephrine to the solution surrounding the artery and in the other, on the contrary, they were completely relaxed with No-Spa (structural analogue of papaverine). The top curves in Figure 3a and 3b show that, in the latter case, normotensive and hypotensive arteries distended readily already at low pressure, but they became very rigid at pressures in excess of 80 mm Hg (their diameter virtually failed to enlarge any further). The shape of the distension curves is the same; there were no qualitative differences in distensibility of normotensive and hypotensive arteries with complete relaxation of myocytes.

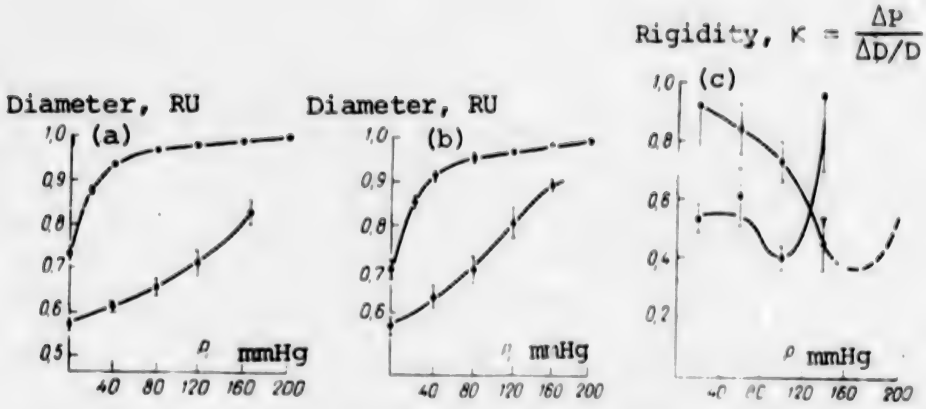

Figure 3. Outside diameter (y-axis, RU [relative units]) of caudal artery as a function of distending pressure (x-axis, mm Hg) in control rats (a) and rats with hypotension lasting 30-60 days (b)

c) rigidity coefficient ($P/\Delta D/D$) as a function of distending pressure (x-axis, mm Hg) in control rats (1) and hypotensive rats (2)
Y-axis--coefficient of rigidity ($10^6 \cdot$dyne/cm^2)

The situation is different when myocytes are activated with norepinephrine in a concentration of 1 μg/mℓ. The amplitude of the restricor reaction with such a concentration constitutes about 50% of the possible maximum [8], and such activation can be considered average. In this case, the shape of the distension curves is qualitatively different. With increase in pressure, there is inreasingly steep growth of diameter of normotensive arteries. At first the shape of the distension curve for hypotensive arteries is the same, but when pressure exceeds 120 mm Hg it changes: there is a tendency toward decreased distensibility of arteries or, in other words, increase in their rigidity.

The quantitative characteristic of rigidity of a vessel as a structure is the ratio of pressure increment to relative increase it causes in diameter, the coefficient of rigidity. If its value for normotensive and hypotensive arteries is plotted against pressure (Figure 3c), the quantitative differences in their behavior as distension increases are quite graphically demonstrable. The rigidity of normotensive arteries decreases constantly, while that of hypotensive arteries first decreases, but then increases significantly in the range of normal physiological pressure (100 to 140 mm Hg).

In order to comprehend why there is a decrease in rigidity of a small muscular artery when it is distended, it is sufficient to imagine the behavior of a rubber-like tube: as it becomes distended there is decrease in the ratio of wall thickness to inside radius, i.e., the geometric factor that determines rigidity of the tube as a structure. If distension of a normotensive artery with activated muscle sheath is continued by applying high pressure, the capacity of this sheath to resist distension will be depleted, and the vessel will require the support of the passive skeleton. According to the curve of distension of a relaxed artery (see Figure 3a and 3b), when the skeleton assumes the entire load and it is tense, its rigidity is quite high. For this reason, the

rigidity of an activated normotensive artery at high pressure will begin to grow, but now because of involvement of the skeleton, and this is shown by the dotted line in Figure 3c. Consequently, when hypotensive arteries are distended, their muscle sheath ceases to counteract distending pressure even in the physiological range of pressures, and for this reason the support of the skeleton becomes involved earlier. This means that the muscle sheath of such arteries resists less to the distending effect of normal pressure (see Figure 3c).

Having determined this, one can predict that the responses of hypotensive arteries to a constrictive stimulus will begin to weaken at lower pressure than the responses of normotensive arteries. Figure 4a illustrates the method of estimating the amplitude of such responses. The corresponding values in the bottom curve (2) characterizing distensibility of the same arteries with some additional activation of myocytes with norepinephrine are subtracted from the values in the top curve (1) reflecting distensibility of arteries due only to myogenic tonus of myocytes. The difference is the amplitude of constrictive responses at a given degree of activation and under different pressures.

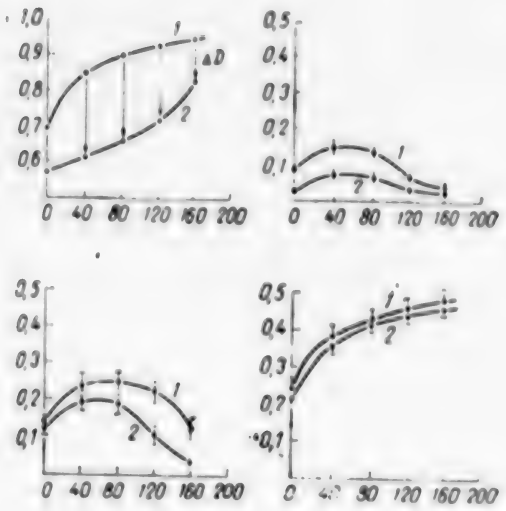

Figure 4.
Method (a) and results of calculating constrictive reactions of arteries (y-axis, relative units) activated with norepinephrine in concentrations of 10^{-7} (b), 10^{-6} (c) and 10^{-5} (d) g/mℓ at different distending pressures (x-axis, mm Hg)
a: 1) initial state of artery
2) activated artery
b-d: 1) control
2) hypotension

[Translator's note: source does not show letter designations of figure sections.]

Figure 4b clearly shows the tendency toward decrease in constriction of mildly activated hypotensive arteries over the entire range of pressures; Figure 4c shows that, at pressure in excess of 80 mm Hg, the estimated constrictor responses of hypotensive arteries in a state of average activation are reliably lower than for normotensive arteries. When, however, normotensive and hypotensive arteries are activated to the maximum extent, they undergo virtually the same degree of constriction at any pressure (Figure 4d). This means that the muscle sheath of hypotensive arteries still remains strong enough for very marked activation to lead to virtually complete closure of the lumen. Thus, changes in the muscle sheath of hypotensive vessels could be concealed when there is strong activation of myocytes, but they must be manifested in the form of less constriction in case of mild and average activation.

This conclusion is confirmed by a direct comparison of extent of constriction of normotensive and hypotensive arteries by norepinephrine: at concentrations of 0.3-1 μg/mℓ, i.e., with average activation of myocytes, the hypotensive arteries constrict about one-third less than normotensive ones [8]. Both are

distended at the same pressure, 100 mm Hg, but for hypotensive arteries this corresponds to the conditions that would be present in man upon restoration of hydrostatic pressure to deconditioned vessels of the legs.

Let us return to the question of whether or not there is change in geometric dimensions of vessels in the presence of chronic hypotension. It is obvious that a comparison of these dimensions in normotensive and hypotensive arteries is relevant only if strictly identical conditions are provided. This could refer to total relaxation of arteries and marked tension of the wall. The latter is achieved by raising pressure to 200 mm Hg. In this state, the outside diameter of hypotensive arteries (30-60-day hypotension) was an average of 10% smaller than that of normotensive arteries [862±31 μm (n = 8) and 962±26 μm (n = 10); $P<0.05$]. The cross section of the wall decreased by about 15%, while relative wall thickness did not change. Thus, in the presence of hypotension there is development of a minor proportionate decrease in geometric dimensions of small arteries. Of course, the correlation between dimensions could be different for other states. For example, at pressure of 100 mm Hg the outside diameter of normotensive and hypotensive arteries with average activation is the same, a mean of about 650 μm. This can be attributed to the fact that the latter are more distensible in this state, although they are somewhat smaller. The estimate shows that, under the conditions described above, the wall of hypotensive arteries must be about 25% thinner. This applies, however, to a small artery and, for this reason, is only indirectly indicative of the possibility of structural alteration of arterioles when pressure is low for a long time.

In conclusion, let us try to conceive of the consequences to which deconditioning of resistive vessels should lead with respect to orthostatic tolerance of circulation. They perform a function of utmost importance in the system that controls arterial pressure: they determine the rate, at which blood "escapes" from the arterial system into the venous one. There is a small volume of blood in the arterial system. For this reason, even a minor increase in rate of escape of blood from some part of the systemic circulation into the veins could have terrible consequences if it is not balanced with sufficient precision by a decrease in such escape from other parts and/or increased delivery of blood from the heart: arterial pressure drop beyond the limit that is hazardous to supplying the brain. This is why control of the state of resistive vessels, these regulators of velocity of arterial blood flowing into the low-pressure system, must be particularly reliable, when a rather large volume of blood accumulates in capacitive vessels of lowered legs and minute volume decreases in orthostatic position [1, 18].

Nevertheless, when we stand, walk or run, the functioning muscles need more blood and they force their resistive vessels to dilate: an area of drastically accelerated displacement of arterial blood into veins appears there. At the same time, arterial pressure does not drop and we do not lose consciousness by any means. This is due to the fact that the signals of functioning muscle receptors elicit corrective reflex reactions, which prevent the potentially possible arterial pressure drop acting on the principle of regulation according to perturbation [11].

When there is restoration of the effect of hydrostatic forces on resistive vessels of dropped legs that have become deconditioned by the absence of these forces, there should also appear a region of more intensive escape of blood into veins. In fact, the muscle sheath of such vessels resists less to distension, and this capacity depends largely on the support of the passive skeleton. Its active myogenic mechanism, which strives to attenuate the distending effect of intravascular pressure and stabilize the vascular lumen, is weakened or even entirely depressed. Finally, there is decrease in amplitude of reactions of resistive vessels to constrictor neuron impulses. All this could create a "weak point" and require intensification of reflex mechanisms to maintain arterial pressure.

Yet, in this instance unlike the one with muscular contraction, reflex mechanisms of control in advance of total peripheral resistance and intensification of cardiac function are not triggered, and for this reason decline of arterial pressure begins with any significant acceleration of escape of blood into the veins. The baroreceptor system should come into pla ut, like any feedback system (by virtue of its very principle), it can affect the controlled parameter only after there is a deviation in it, i.e., when arterial pressure already starts to drop.

Ultimately, the success of reflex control is determined by the effector element. Deconditioned resistive vessels are less effective regulators of blood flow and, in orthostatic position, they could become the element that initiates disturbances in resistance of the cardiovascular system, the area of accelerated "discharge" of blood from the arterial system.

When discussing the mechanisms of orthostatic intolerance, the opposite situation is usually considered, i.e., diminished influx of blood to the heart and decreased delivery of blood to arteries, and for this reason the venoconstrictive reflexes are called the "first line of defense" [15]. There are no grounds to engage here in the debate that is going on concerning this issue [16, 18, 21], although we do have data to the effect that prolonged decline of pressure in capacitive vessels of the limbs diminishes their capacity to withstand distension [9] and that this capacity depends more on tonic activity of the vasoconstrictor system [10]. Using the same metaphor, "first line of defense," it can be stated that this line is maintenance of normal properties of both resistive and capacitive vessels, which is achieved by the conditioning effect of intravascular pressure, including its hydrostatic component.

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HEMOPOIESIS IN RATS SUBMITTED TO WEIGHTLESSNESS

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[Article by V. N. Shvets, A. Vacek, G. I. Kozinets, I. I. Britvan, V. I. Korol'kov and N. A. Chel'naya (USSR and CSSR)]

[English abstract from source] This paper summarizes experimental data on the erythropoiesis of rats flown on Cosmos biosatellites for 18-22 days. The histogenesis of the hemopoietic tissue is investigated at the level of stem cells, dividing-maturing pool and mature blood cells (erythrocytes). In weightlessness inhibition of the erythropoiesis in various skeletal sites occurs. Flight data are compared with hemopoietic findings in hypokinetic rats. Possible mechanisms underlying red blood disorders in humans during spaceflight are discussed.

[Text] Determination of the genesis of hematological changes, which have been consistently observed in spacecraft crews since the mission of Gemini-5 has been a part of the program for Cosmos series biosatellites. The earliest results, which were indicative of reliable decrease (by 10-20%) in erythrocyte mass, were attributed to the effect of the hyperoxic environment of Gemini and Apollo spacecraft. The initial assumption that oxygen was the main etiological factor in this phenomenon was not confirmed, since erythrocyte mass diminishes aboard the Skylab orbital station (under normoxic conditions). It was noted that this effect was much more marked during short-term missions (maximum decline observed on 20th to 40th flight days) [15]. Subsequently two hypotheses were advanced concerning the mechanisms of impairment in erythrocyte count. The first assumed that there were changes in metabolic processes in the erythrocyte population itself. However, studies in this direction showed that no serious metabolic changes are observed in erythrocytes [9, 10, 14]. Leon et al. [12], who studied the parameters of erythrocyte survival in rats flown in Cosmos-782 biosatellite, established that the life span of erythrocytes diminished inflight, while hemolysis tripled, as compared to the control. Probably, one of the causes of erythrocyte loss in weightlessness could be their hemolysis due to premature aging of part of the erythrocytes and decrease in their resistance. There are not enough data to make a definitive judgment on this score. On the whole, the accumulated material leads to the conclusion that the decrease in erythrocyte mass is not related to impairment of their structure and function. According to the other hypothesis, loss of erythrocyte mass is due to inhibition of erythropoiesis.

Our objective here was to sum up the findings of studies of the condition of blood of rats flown aboard biosatellites of the Cosmos series and used in model experiments. It did not include a description of the different results submitted in a number of publications [2-4, 8, 11, 16].

Studies in Weightlessness

All rats presented a statistically reliable lag (by 9-10%) in weight gain, as compared to control animals, during the period of spaceflight (Cosmos-605, Cosmos-782, Cosmos-936 and Cosmos-1129 biosatellites). Perhaps this was partially attributable to the nutritional factor, but most likely to the adverse effect of spaceflight on growth of tissues and organs.

The cellular composition of peripheral blood changed substantially several hours after landing (Cosmos-936, Cosmos-1129). There was leukocytosis, lymphopenia and neutrophilia, which are inherent in the acute period of the stress reaction. These parameters rapidly returned to the control level by the 3d postflight day. Minor changes also occurred in the erythrocyte population: decrease in number of discocytes (by about 8-10%), appearance of altered forms--echinocytes (5.6%) and spherical erythrocytes (2.3%). Such erythrocytes were not found in control animals. At the same time, mean dry mass of erythrocytes was normal at all tested times. However, erythrocytes differing in size and hemoglobin content were dissimilarly distributed. On the 3d postflight day, there was an increase in number of erythrocytes with greater hemoglobin content. Osmotic resistance of erythrocytes underwent the greatest change: resistance to hypotonic solution decreased by 44%, as compared to the control. All other clinical parameters of blood were normal.

Thus, a similar erythrocyte reaction--discocyte-echinocyte transformation--was noted in animals and man [11]. There were only insignificant quantitative changes in the erythrocyte population, manifestations of a stress reaction accompanied by change in leukocyte count and, perhaps, some erythrocyte properties.

The very first studies of histological structure of marrow in long bones of the posterior extremity of rats (Cosmos-605 and Cosmos-782 biosatellites) revealed depression (by about 50%) of the erythroid stem cells with retention of normal general mitotic activity of karyocytes. These data were confirmed by Ellis et al. [8], who observed a decrease in number of erythroid cells in rib marrow and sternum of mice flown in Apollo. In addition, complete depression of erythropoiesis was also demonstrated in the spleen of flight animals [1].

Inhibition of erythropoiesis was also observed in rats flown aboard Cosmos-936. Myelograms of bone marrow of the femur and humerus of flight animals showed statistically reliable increase in number of undifferentiated (blast) cells and total absence of reticular cells. At the same time, the number of all myeloid elements increased by about 32 times, as compared to the number of such cells in the control group of animals. As a result, the ratio between number of erythroid and myeloid cells was shifted in the direction of increased myelopoiesis.

On the 25th-27th postflight days, all of the above parameters reached a normal level.

Thus, the results of differential count of different categories of cells in the dividing-maturing pool of flight rat bone marrow are generally indicative of inhibition of erythropoiesis, intensification of granulocytopoiesis and appearance of undifferentiated forms of cells. The latter indicates that hemopoiesis in bone marrow was shifted in the direction of rejuvenation. Since attenuation of erythropoiesis occurred in different parts of the skeleton and spleen, it can be assumed that the effect of spaceflight factors is transmitted through the system that regulates erythropoietic activity. Is there a cause-and-effect link between inhibition of erythropoiesis, which was noted in the animals, and decrease in erythrocyte mass, which was observed in cosmonauts? The answer is not clear as yet, since it is difficult to extrapolate to man the data obtained for animals.

It is known that histogenesis of hemopoiesis is based on proliferation and differentiation of pluripotent stem cells that provide the diversity of cell forms with different functions.

The first studies (by the method of Till and McCulloch) of stem cells (colony-forming units--CFU) of rats flown aboard Cosmos-605 failed to demonstrate any appreciable deviations, either in quantity or CFU capacity for differentiation in the direction of the three main myelopoietic elements. The results of the second experiment on rats flown aboard Cosmos-936 were just the opposite of the preceding one. It was established that there was a decrease to 1/20th in absolute number of CFU in humeral marrow, while proliferative activity (according to oxime-urea "suicide" test) decreased to nil, versus 28% in the control. Using the cloning method, it was found in the spleen and bone marrow of the recipient that CFU differentiation along the route of formation of erythroid cells was substantially inhibited (by 3-4 times), while granulopoiesis increased by 2-3 times, as compared to the control.

Thus, there was marked decrease in flight rat CFU capabilities in the direction of erythropoiesis, while granulopoietic capacity increased, which is consistent with the results of studies of the dividing-maturing pool of bone marrow cells of these rats.

The contradiction of data obtained about animal CFU properties can be attributed to the fact that the biological material was collected and examined at different times. The bone marrow from rats flown aboard Cosmos-605 was examined 2 days after the flight. In this time, there could already have been restoration of number and differentiation of CFU. For technical reasons, the bone marrow of rats flown aboard Cosmos-936 was stored under refrigeration for 4-5.5 days before being transplanted to a recipient. Consequently, the possibility cannot be ruled out that the CFU of flight animals could have diminished physiological resistance to long-term storage, as compared to resistance of control animal cells. It is known that duration and conditions of storing bone marrow affect the quantitative characteristics of the CFU population, but not qualitative ones [13].

It is difficult to determine at this time whether spaceflight factors affect the properties of the CFU population, since the sparse data available on this score are quite contradictory.

Thus, a decrease in erythrocyte mass is observed in man during spaceflights; in animals, there is inhibition of erythropoiesis on the systemic level; the period of readaptation to earth's gravity is characterized by restoration of erythrocyte mass and erythropoietic activity.

On the whole, however, the problem of space hematology is still at the stage of inception and, consequently, it requires accumulation of hard facts and solution of a number of problems. For example, we do not know which mechanisms cause inhibition of bone marrow activity in the erythroid direction, insufficient load on the muscle, stress, possible restriction of fluid and food intake by animals, or general decline of metabolic processes in the body. Nor do we know at which level of histogenesis of hemopoietic tissue does impairment of erythrocyte reproduction occur in man (if this happens) and what role could be played in this by the sequelae of redistribution of blood, dehydration of the body and humoral regulators of erythropoiesis. However, whatever the causes of change in the "steady state" of hemopoietic functions, it must be conceded that the data already available concerning erythrocyte mass and erythropoiesis reflect physiological adaptation of erythron to weightlessness, rather than a pathological process.

Current conceptions of histogenesis of hemopoietic tissue enable us to expound two hypotheses. According to the first one, there is first a change in weightlessness on the level of committed (rather than stem) cells that are sensitive to erythropoietin (erythropoietin-sensitive cells--EPSC). The decrease in activity of the latter leads, in turn, to attenuation of erythropoiesis on the level of the dividing-maturing pool, which is ultimately associated with decreased erythrocyte reproduction. It is a known fact that the triggering mechanism of change in EPSC activity is erythropoietic, which reacts to changes in partial oxygen tension in organs and tissues. If we consider that, in the presence of slower anabolic processes in weightlessness oxygen requirement diminishes, this mechanism does indeed exist. The second and oldest is the feedback mechanism: if there is an excessive volume of erythrocyte mass in weightlessness, in relation to ground-based conditions, this should lead to hemolysis of some erythrocytes, and this is apparently what occurs in cosmonauts. Finally, the above mechanisms could be present concurrently, independently of each other.

Model Experiments

Additional experiments were conducted on mice and rats under conditions simulating some spaceflight factors in order to check the validity of the opinion that an insufficient muscle load plays a part in depressing erythropoiesis in weightlessness. As such a model, hypokinesia was chosen, which simulates the insufficient dynamic load in weightlessness. Evaluation (on myelograms and histologically) of cellular composition and structure of rat femur bone marrow revealed substantial quantitative changes in the population of young erythroid cells. The number of the latter changed uniformly. For the first 2 weeks of the experiment, there was an increase (by 2 times) in number of erythroid cells followed (on the 60th day) by 50% decrease, i.e., just as was found after a 3-week spaceflight. Analogous experiments were conducted with mice in order to study the kinetics of CFU number and differentiation. Mice were used because rat CFU did not satisfy the requirements for pluripotent stem cells as well as mouse CFU, and were more referable to committed cells with regard to their properties [6, 7].

The results indicated that there was a 2.5-fold increase in absolute CFU of bone marrow in the 1st week, after which (and up to the 45th day of observation) it decreased to control values or exceeded them somewhat, whereas CFU concentration and total number of karyocytes remained on the control level. The described quantitative changes were not related to the nature of distribution of transplanted CFU in the recipient, since the settling factor (parameter F) remained constant at all stages of observation and did not differ from the control.

Thus, no general pattern was demonstrable after comparing the quantitative changes in the CFU population under hypokinetic and weightless conditions. Evidently, in each experimental situations (weightlessness and hypokinesia) there were different mechanisms involved that affected reactivity of the CFU population and, in general, histogenesis of hemopoiesis. Consequently, the muscle load deficiency, which was present during hypokinesia, is not the prime factor altering the quantity and distribution of CFU in the body in weightlessness. This conclusion is confirmed by the data obtained from a study of CFU differentiation. The erythropoietic properties of CFU were not impaired under hypokinetic conditions, with the exception of the first 2 weeks of the experiment, when the capacity fo CFU to form erythroid precursors increased by about 2-3 times. The results of subsequent experiments revealed that this effect, like the change in CFU resistance, was due to redistribution of T lymphocytes, their migration into bone marrow due to development of a stress reaction [4, 5].

The submitted results concerning restriction of motor activity of animals did not basically coincide in either time or nature of changes with the data obtained in the study of the CFU population in rats during the experiments aboard Cosmos-605 and Cosmos-936 biosatellites. The only exception was depression of erythropoiesis on the level of the dividing-maturing pool of rat bone marrow cells after hypokinesia. However, even in this case, the time of development of this process differed markedly: 60 days of hypokinesia corresponded to 18-22 days of weightlessness. It may be that the model of clinostatic hypokinesia is not strictly adequate for investigation of hemopoietic disorders that occur in weightlessness.

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EXPERIMENTAL AND GENERAL THEORETICAL RESEARCH

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POTENTIAL USE OF DIALOGUE WITH COMPUTERS IN BIOMEDICAL RESEARCH

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 18, No 4, Jul-Aug 84 (manuscript received 21 Apr 83) pp 16-19

[Article by T. M. Smirnova]

[English abstract from source] Problems involved in the development of interaction software for biomedical investigations and in the preparation of experimenters for using a computer in the interactive mode are discussed. An interaction program for data analysis is suggested. The program is used to build an interactive modification that makes it possible to carry out a computer-aided verification of the mechanisms of erythropoiesis regulation in spaceflight. It is shown that a simple interactive terminal facility can be employed in the software of biomedical investigations, taking into account changes in the requirements placed by users and their experience of computer interaction.

[Text] At the present time, man-computer dialogue is gaining new areas of application and opens up unlimited possibilities for improving the efficiency of studies that involve analysis of a large amount of scientific information. This matter is of particular interest to modern biology and medicine, especially space biology and medicine. The complexity of living organisms, the high degree of their dependence on the environment, complex nature of influence on the body of spaceflight factors--all this requires use of computers on any level of research in this area. The need to develop dialogue systems (DS) oriented toward consumers--biologists and medical workers--is also attributable to the fact that knowhow in communicating with a computer in the dialogue mode usually helps consumers gain better understanding of the formulation of their own tasks and realization of their information needs. This is indicated by the experience with using DS in the most varied problem areas [12]. Interaction with computers of representatives of different scientific disciplines stimulates development of a common language and methodology of research, as well as formulation of objectives of interdisciplinary studies. It would be difficult to exaggerate the significance of this process to such a complicated and multifaceted area as space biology and medicine.

The DS used in biomedical research can be divided into two main classes according to their purpose. The first class consists of reference information systems. DS of this class have been rather well developed theoretically at the present

time [4] and they are used in research practice. The second class refers to DS that make it possible to automate time-consuming calculations. This report deals with problems of developing the second class of DS. We shall discuss two areas of application of DS--analysis of experimental findings and analysis of complicated hypotheses using simulation models.

Data processing is a mandatory stage of any experimental study. This very fact requires development of software that can be used for an experimenter having no training in programming. During long-term complicated experiments, which are typical in space biology and medicine, the need for means of ongoing analysis of recorded information increases, particularly because of the need to correct the plan of an experiment in order to avoid inadmissible changes in state of the organism studied or compensate for unforeseen changes in the environment. At the present time, development of DS for data analysis is proceeding in two directions. The first direction is related to organizing dialogue of the "initiator--computer" type, where the computer poses questions, while the user's role amounts essentially to selecting the required variant of the set of answers (so-called menu) offered by the computer. The most interesting Soviet work in this direction are the studies of G. R. Gromov and M. A. Roytberg [9, 10, 11]. The second direction, which is being developed by R. E. Asratyan et al., is based on organizing dialogue of the "initiator--man" type [1, 2]. In this case, the user prepares a program for the computer in a special language, of the directive type, which is of utmost simplicity, for the study and adapted to formulate the purposes of processing medical information. There are several reasons for the limited use of the above-mentioned DS in biomedical research. DS that place control of the dialogue with a computer are too narrowly oriented toward a specific customer, and it is impossible to alter the computing part of the DS to conform to the needs of new users without the help of a highly skilled programmer. The obstacles to mass scale use of dialogue with a computer, initiated at least in part by man, are related to the need for serious prior training of users. The study of the dialogue language is the simplest of the problems that arise. Considerably greater difficulties are related to development in users (biologists and medical workers) of an algorithmic approach to formulation of problems. Its formation is a special problem, the solution of which could be aided by use of simpler forms of dialogue.

It is apparent from the foregoing that development of DS should have the purpose not only of facilitating the work of a researcher with regard to converting and analyzing information, but training nonprofessional computer users in perception and use of new investigative methods related to the use of computers. It must also be borne in mind that accumulation of knowhow in independent work with a computer leads to a change in the users' conceptions of optimum organization of a dialogue. Evolution of DS specifications is oriented in the direction of expanding the set of problems that can be solved, rendering the dialogue language more similar to natural language, from the most elementary variants of dialogue initiated by a computer to dialogue initiated by man [3, 8]. Taking this into consideration, we can suggest the following program for development of DS and training researchers in the fields of biology and medicine to work with them. At the first stage of familiarizing users with a computer, it is desirable for them to learn to use some "base" general-purpose DS, which use a simple

dialogue structure controlled by the computer. Further development of DS should be based on the requirements advanced by users as they accumulate experience in communicating with computers, and it should take into consideration the inevitable refinement of user training in the area of mathematical methods.

We have developed a dialogue program for data processing as a "base" DS that could serve as the first step on the way toward mastering dialogues with a computer. The computing part of the program makes it possible to perform the most common statistical procedures in biomedical research: calculation of mean, dispersion and standard deviation of mean sample, calculation of t criterion for pairs of samples, coefficient of correlation and value of t criterion for it, coefficients of linear regression, as well as construction of sample histograms. There are provisions for checking and correcting the base digital information and separating the base samples into subgroups. The program is written in the FORTRAN language and executed on an SM-4 computer in the operational system OS-RV. A display is used to exchange messages between the user and computer. A printer puts out the results of calculations and, if necessary, prints out the base information. The procedures for inputting and outputting data, making corrections and calculations can be alternated in any desired order. In the event of onset of an erroneous state, the user is instructed as to the necessary corrections. The structure of the program permits easy alteration of the set of computing algorithms, modes of input and output, and correction of data. Changes related to addition of new subprograms and work with the program do not require any special training in computer programming or use.

The data processing program has gained rather wide use among researchers--physiologists, biochemists and psychologists. It is enough for representatives in these special fields to hav. one practice session lasting 1-2 h in the presence of a consultant-mathematician to learn to work with the program. Laboratory technicians easily learn to process data in accordance with a given program. Experience in using the program made it possible to define the next tasks in the area of developing programmed dialogue support for a number of experimental studies. Independent work with the program sometimes stimulates users to search for a way to make a more thorough statistical and logical analysis of data. In the course of preparing data for processing, the experimenter has the opportunity to assess the influence of the program of experimental measurements (number of measurements and their distribution in time) on level of significance of formulated conclusions. This leads us to expect that the use of even the most elementary DS in research practice will be instrumental in disseminating the basic ideas of experiment planning theory, as well as in developing this theory in accordance with the requirements of medical and biological research.

The principles for organizing a dialogue program could be extended to programs designed for analysis of complicated hypotheses using simulation models if one uses as the set of computing algorithms a set of alternative variants of models of the object under study and exogenous effects on it. Organization of work with models in the mode of a dialogue with a computer would make it possible to involve specialists, playing the part of model users, in the process of construction and analysis of the models. Participation of experimenters would increase the effectiveness of modeling significantly; without him it would be

impossible to convert simulation models into a real tool in experimental and theoretical research.

In the general case, organization of dialogue for work with models at a pace that is suitable for the user requires highly productive computers. However, it is permissible to use low-power computers on the level of refining the different subsystems of complex models. Participation of specialist-experimenters in the modeling process is of greatest importance at expressly this stage, i.e., on the level of selecting hypotheses placed in the model. We developed a dialogue program that produced on an SM-4 computer a simulation model of erythropoiesis regulation during a spaceflight and experiments that simulated it. The computing algorithm was based on the model of population dynamics of erythrocytes [6]. The model simulates well the decline of erythrocyte mass and its restoration after spaceflights lasting up to 3 months, as well as the dynamics of erythropoiesis during long-term hypokinesia [5]. However, when we tried to simulate the dynamics of restoration of erythropoiesis following particularly long flights, it was found necessary to expand the class of hypotheses concerning the mechanisms of controlling this system in weightlessness. The results of several experimental studies [7, 13, 14] revealed that adaptation of erythropoiesis to spaceflight conditions involves the structure and metabolism of erythrocytes. There were provisions in the model for consideration of the effect of these factors on efficiency of gas transport by blood. Interrogations of the computer about the appearance of the function of efficiency of gas transport and other exogenous inputs of the model were formulated in a form that is comprehensible to the experimenter-user who is not familiar with the mathematical aspect of the model. With this model it was possible, in particular, to demonstrate that the reduction in average erythrocyte diameter, which is observed during long-term flights, can affect the level of decrease in erythrocyte mass during spaceflight and rate of its postflight recovery.

These examples are indicative of the vast potential of using the most elementary means of dialogue with a computer in biomedical research and development, on their basis, of program support for research in this field, with consideration of the dynamics of user interrogations and their experience in communicating with computers.

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VITAMIN METABOLISM IN COSMONAUTS FOLLOWING SHORT-TERM FLIGHTS

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[Article by M. S. Belakovskiy, N. D. Radchenko and N. G. Bogdanov]

[English abstract from source] The vitamin status of the cosmonauts after short-term (4 to 13 days) flights showed different variations. Vitamin consumption was basically adequate to the requirements in spaceflights. Some vitamins were occasionally in deficiency, thus indicating their enhanced metabolization.

[Text] Extreme states are associated with increased utilization of many vitamins, change in their interstitial metabolism and differentiated change in the body's vitamin requirements. The results of different studies are indicative of a change in vitamin metabolism and utilization in the presence of adaptation stress when performing aircraft and space flights [1, 2, 6, 7]. We shall submit here the results of a study of vitamin metabolism before and after short-term flights (4-13 days) aboard Soyuz spacecraft and Salyut-6 orbital stations.

Methods

In order to evaluate cosmonauts' vitamin status (thiamine, riboflavin, niacin, pyridoxine, A and carotene, cyanocobalamin, α-tocopherol and D_3), conventional criteria were used, which determine the vitamin balance in the body: vitamin content of food allowance, their concentration in blood, excretion of vitamins or their metabolites in 24-h urine, activity of enzymes, the coenzyme part of which contains vitamins, distinctions of enzyme activation by their coenzymes.

Thiamine status was assessed by the activity of erythrocyte transketolase and its increase after addition of thiamine diphosphate (TDP effect) [5], as well as 24-h thiamine excretion in urine [16]. Riboflavin status was determined on the basis of parameters of glutathione reductase (GR) of erythrocytes and its increase after addition of flavin-adenine dinucleotide (FAD effect) [16] and daily excretion of riboflavin in urine [4]; niacin was assessed by levels of pyridine coenzymes (NAD + NADP) in blood [14] and excretion of N_1-methylnicotinamide in urine [11], vitamin B_6 was evaluated on the basis of excretion of 4-pyridoxylic acid [12], activity of erythrocyte aspartate transaminase and level

of the pyridoxal phosphate effect [13]. Concentrations of vitamin A, carotene, cyanocobalamin, α-tocopherol and basic transport form of vitamin D_3--25-hydroxycholecalciferol (25-OH-D_3) were assayed in blood serum [8, 10, 13, 17]

Vitamin excretion in urine was assayed 1 month and 1-5 days before the spaceflights and for the 1st to 5th days after them. Venous blood, which was drawn on a fasting stomach, was analyzed 1 month before spaceflights and on the day after their termination.

Results and Discussion

The study of vitamin status of cosmonauts who were undergoing intensive training for flights aboard craft of the Soyuz type and who worked on the Salyut-6 orbital station revealed that the average data obtained 1 month before the missions did not exceed the conventional norm for healthy males in all parameters. The noted individual fluctuations of some parameters were apparently related to some natural differences in diet and irregularity of physical and mental loads during the training period. In some cases there was a moderate deficiency in thiamine, riboflavin, pyridoxine and niacin (according to excretion in 24-h urine and results of testing enzyme activity and degree of their activation by the appropriate coenzymes). We observed a relatively high serum vitamin A level, 53.4±3.1 μg% (its concentration was at the bottom of the normal range, 27.4 μg% only in the flight engineer of Soyuz-37), as well as carotene--153.1 μg% (2 samples out of 17 showed levels at the bottom of the normal range) and cyanocobalamin--717.1±64.5 pg/mℓ (2 samples out of 15 were at the bottom of the normal range). In all cases, blood concentrations of 25-OH-D_3 and α-tocopherol were relatively high (average 51.2±5.4 ng/mℓ and 1.47±0.10 mg%, respectively).

Table 1. Level of vitamin excretion in 24-h urine before and after short-term flights (n = 23)

Parameter	Time before flight		Postflight day		Normal physiological ranges
	1 month	1-5 days	1	2	
Thiamine, μg	318.1±37.2	532.3±86.9	284.6±35.1	310.2±36.3	150–500
Riboflavin, μg	352.6±31.5	710.2±92.8	342.9±91.6	433.6±52.4	300–1000
4-Pyridoxylic acid, mg	2.54±0.23	4.64±0.37	2.87±0.54	2.69±0.52	1.5–2.5
N_1-methylnicotinamide, mg	10.3±0.5	12.93±0.93	10.85±1.16	10.04±0.80	7.0–12.0

It was recommended that the cosmonauts take a vitamin and amino acid complex preventively each day for 2 weeks prior to a spaceflight; the complex consisted of retinol acetate, α-tocopherol acetate, thiamine bromide, riboflavin, pyridoxin, folic acid, rutin, methionine, cyanocobalamin, ascorbic acid and nicotinamide.

Table 2. Parameters of vitamin metabolism in cosmonauts' blood before and after short-term spaceflights

Parameters	Number of subjects	Preflight	Postflight	Normal physiol. range
Vitamin A, μg%	17	53,4±3,1	60,8±4,5	30 70
Carotene, μg%	17	153,1±11,9	178,8±11,6	80 230
Vitamin B_{12}, pg/mℓ	15	717,1±64,5	619,2±59,3	200 900
Vitamin D_3(25-OH-D_3), ng/mℓ	15	51,2±5,4	58,6±4,6	10 100
Vitamin E, mg%	15	1,57±0,10	1,48±0,07	0,6—1,6
NAD+NADP, μg/mℓ	13	30,6±4,1	29,7±3,3	28—44
TDP effect, %	13	14,9±3,5	12,5±1,03	<15
FAD effect, absolute units	11	1,03±0,02	1,08±0,03	<1,2

The preflight diet included a wide assortment of vitamin-rich fruit and berry decoctions (dog rose, dried apricots, etc.), vegetables and fruit. For this reason, control tests of 24-h urine in this period showed a substantial increase in concentrations of vitamins and their metabolites. For example, thiamine excretion increased by 67.3%, riboflavin by 101.4%, N_1-methylnicotinamide by 25.5%, 4-pyridoxylic acid by 82.7% (Table 1). No blood tests were done in this period.

The results indicate that the cosmonauts' vitamin status was adequate during the period of intensive training immediately preceding spaceflights.

The cosmonauts had a previously described diet during the flights aboard Soyuz series craft and when working on the Salyut-6 orbital station [3].

Postflight excretion of vitamins and their metabolites was characterized by a wide amplitude of fluctuations and periodicity. The periods of fluctuations were individual in nature. In some cases there was drastic decrease in daily excretion of vitamins beyond the bottom of the normal range (for example, thiamine and riboflavin).

Thus, in the commander (CDR) of Soyuz-36, riboflavin excretion in urine constituted 94.8 μg on the 1st postflight day and 202.8 μg on the 2d day; for the flight engineer (FLE), the figures were 85.2 and 109.2 μg, respectively. Blood tests revealed that the FAD effect constituted 1.28 and 1.27 absolute units in the CDR and FLE, respectively and GR constituted $11 \cdot 10^{-3}$ and $8 \cdot 10^{-3}$ μmol NADP·H_2/million erythrocytes/h. Similar changes were observed in the CDR of Soyuz-T-2, in whom the FAD effect was 1.17 absolute units and GR activity constituted $11 \cdot 10^{-3}$ μmol NADP·H_2/million erythrocytes/h; on the 1st postflight day, riboflavin excretion in urine was 162.0 μg. The same crew member presented some decrease in elimination of N_1-methylnicotinamide (3.2 mg on the 1st day and 6.0 mg on the 2d).

The appreciable decrease in vitamin excretion in urine and their low levels in blood of some cosmonauts are indicative of a high vitamin requirement of the body in this period.

Postflight changes in parameters of vitamin metabolism in cosmonauts' blood were mainly in different directions (Table 2). Statistical processing showed that the differences leveled off. It must be noted that the parameters studied did not usually exceed the normal physiological range. Thus, in 11 out of 17 tested cosmonauts, the concentrations of vitamin A and carotene increased on the average to 60.8±4.5 and 178.8±11.6 μg%, respectively, as compared to preflight data. At the same time, for example, the crew of Soyuz-38 showed a decline in vitamin A content of blood, from 56.7 to 23.6 μg% for the CDR and from 73.9 to 26.8 μg% for the FLE. Similar findings were also made in our analysis of the results of assaying blood concentrations of 25-OH-D_3, cyanocobalamin and α-tocopherol. There was not a single instance where the blood levels of these vitamins had dropped below the physiological norm.

The results of testing the vitamin status of cosmonauts after completing short-term spaceflights lasting 4 to 13 days are indicative of changes in different directions in the parameters examined. This apparently depends on individual distinctions of the body's reactions to the combined effect of flight factors (vibration, noise, levels of physical and mental loads, some natural differences in diet, etc.). At the same time, vitamin intake by the cosmonauts was essentially adequate to the body's requirements under the extreme conditions of spaceflight. However, in a number of cases, there was a deficiency in some vitamins postflight, which is indicative of their intensified metabolization and increased body requirements.

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COMPENSATORY AND ADAPTIVE REGIONAL HEMODYNAMIC REACTIONS TO WEIGHTLESSNESS DURING LONG-TERM SPACEFLIGHTS

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 18, No 4, Jul-Aug 84 (manuscript received 8 Apr 83) pp 22-28

[Article by Kh. Kh. Yarullin, T. D. Vasil'yeva, V. F. Turchaninova, I. V. Sokolova and N. D. Vikharev]

[English abstract from source] This paper discusses regional hemodynamics and vascular regulation during and after spaceflights of over 3 months in duration. Mechanisms of cardiovascular adaptation to weightlessness are described. The postflight differences in the recovery of regional hemodynamics seem to depend on the individual characteristics, age-related changes of the cardiovascular system, as well as the countermeasures and rehabilitation measures performed during and after flight.

[Text] Man's participation in spaceflights is associated with changes in circulation and cardiovascular function [2, 4-6, 13-15]. Rheographic studies of regional and central hemodynamics during and after flights demonstrated significant redistribution of blood in the body and change in vasomotor regulation [7, 10, 13].

This report deals with changes in regional hemodynamics and vascular regulation at rest and with functional loads during and following orbital flight lasting over 3 months.

Methods

A 4RGIM bipolar rheograph was used under basal metabolic conditions to record the rheoencephalogram (REG) in the frontomastoid and bimastoid leads, rheogram (RG) of the right lung and liver, third finger and lower leg. With postural loads, the REG was recorded in the frontomastoid (right) and bimastoid leads, the RG of the lung and leg, as well as photoplethysmogram (PPG) of the third finger, were also recorded. The studies were conducted using an 8-channel electroencephalograph before the flight, on the 1st, 3d, 7th, 12th and 30th postflight days. The inflight REG was recorded using a tetrapolar rheograph, also in the frontomastoid lead, bilaterally. The techniques used have been described in detail in previous articles [1, 10, 11].

Results and Discussion

REG dynamics inflight showed moderate increase in pulsed delivery of blood to the cerebral hemispheres. Thus, on the 14th flight day, the parameter for pulsed filling rose by 39% for the commander (CDR) and by 46% for the flight engineer (FLE), as compared to preflight levels. Yet general electric conduction of the brain increased appreciably more in the CDR--by 67%--versus 60% in the FLE (interelectrode resistance decreased from 92.5 to 38.2 Ω in the former and from 105.5 to 40.4 Ω in the latter). Such increase in general electric conduction of the hemispheres on the 14th flight day is apparently indicative of increase in extravascular fluid in the brain, in addition to filling of blood vessels.

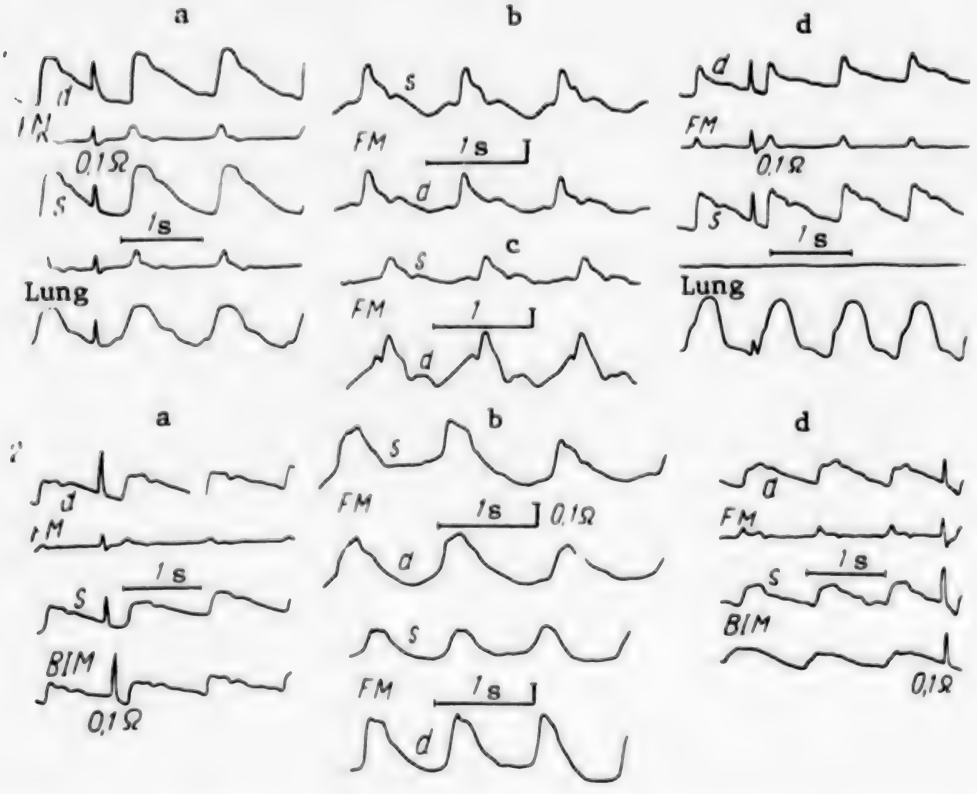

Figure 1. Regional rheograms for CDR and FLE before, during flight and after landing

1) REG of hemispheres (FM) and RG of lung for CDR
2) REG in frontomastoid (FM) and bimastoid (Bim) leads for FLE
a) preflight
b,c) 61st and 93d flight days
d) 1st postflight day
d) on the right
s) on the left

On the 61st day of the flight, filling of both hemispheres was in the normal range (REG amplitude 0.176 Ω on the left and 0.168 Ω on the right); however, it increased significantly (by 93.4 and 47%) in comparison to parameters on the 50th day (REG amplitude reached 0.091 and 0.114 Ω). The shape and significant enlargement of venous waves, from 0.016 and 0.037 to 0.036 and 0.088 Ω (i.e., by 125-137.8%) was indicative of difficult venous efflux from the cranial cavity and dilatation of veins (Figure 1, 1b). As can be seen in this figure, signs of cerebral plethora, particularly the veins, were observed against a background of significant decrease in tonus of arterioles and veins: the parameter for tonus of arterioles and small arteries did not exceed 14.2 and 6.5% and the one for tonus of veins and venules was 24.4 and 28.6%, whereas they reached 61 and 65.8%, respectively, preflight.

Throughout the flight, there was obvious increase in the arterial component of the REG for the CDR, against a background of concurrent decrease of the venous (diastolic) component. This was associated with appearance of marked venous waves on the REG often superimposed on the anacrotic of the next REG wave (Figure 1, 1c), i.e., decrease in venous tonus and difficult venous efflux, particularly from the right hemisphere on the 93d flight day.

Inflight, the FLE showed noticeable increase in both REG components, but more marked for the venous one (see Figure 1, 2b and 2c), most often against a background of elevation and, less often, normalization of indicator of peripheral vascular resistance--ratio of maximum systolic value of venous component to amplitude of arterial component (V/A). On the 14th flight day, the venous waves on the REG reached 0.024 and 0.038 Ω, they disappeared on the 50th day and on the 78th day they reappeared and increased to 0.075 and 0.43 Ω. All this was indicative of plethora of intracranial veins against the background of compensatory elevation [sic] of small precapillary and postcapillary vessels.

Deposition of blood in the veins was apparently involved in the difficult efflux from small resistive vessels, as it altered conditions of transcapillary exchange. In response, there was a compensatory-adaptive reaction by the cardiovascular system, which altered the parameters of pulsed filling with blood in the direction of stabilization of transcapillary exchange.

Hemodynamic adaptation to weightlessness in the CDR was associated with 25% increase in cardiac output [10] and particularly of its cranial component (to 46%, as compared to preflight value) due to intensification of the heart's contractility and suction action (which is also apparent from the high pulse pressure--70 mm Hg). On the REG, this was manifested by a significant increase in amplitude of the arterial component against a background of decline of parameters of cerebrovascular resistance (V/A, DKI [diacrotic index]) and venous tonus (DSI [diastolic index]), i.e., prevalence of active dilatation of intracranial vessels and attenuation of venous pulsation.

Hemodynamic adaptation to weightlessness in the FLE was associated with increase in peripheral vascular resistance aimed at preventing elevation of the head component of cardiac output, i.e., restriction of intracranial plethora. This was manifested on the REG by increase in V/A, DKI, DSI and in amplitude of the venous component, i.e., intensification of venous pulsation against the background of 15% decrease in stroke volume [10].

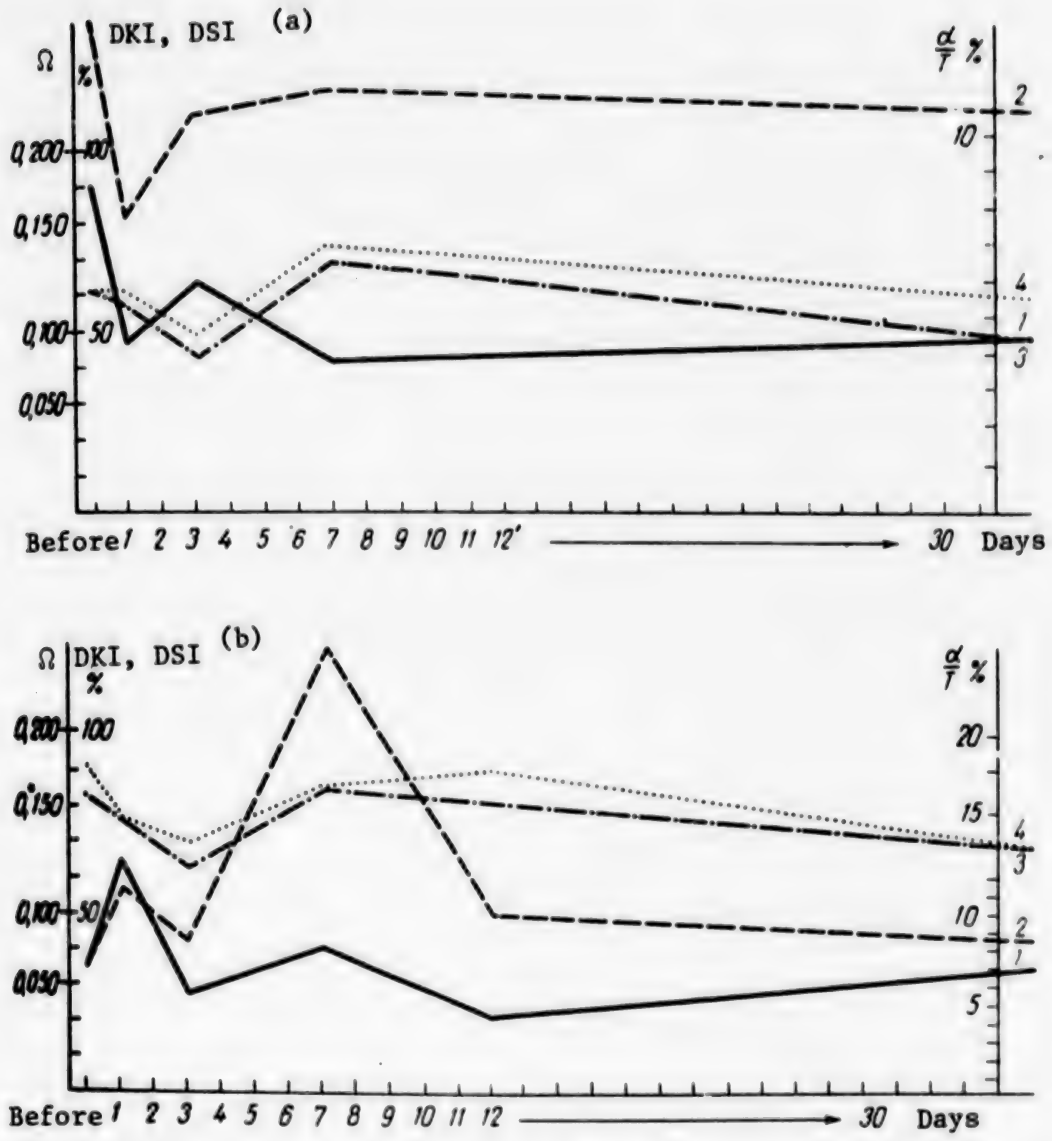

Figure 2. Dynamics of REG parameters in frontomastoid lead before and after flight in CDR (a) and FLE (b) at rest

1) amplitude of REG (in fractions of ohms)
2) α/T%
3) DKI (%)
4) DSI (%)

Use of LBNP [lower-body negative pressure] (-25 mm Hg for 2 min and -35 mm Hg for 3 min) elicited redistribution of components of cardiac output vectors and, accordingly, of volumes of deposited and circulating blood, with which the hemodynamic parameters of transcapillary exchange became virtually normal. On the REG, this was manifested in both cosmonauts by a decrease in amplitude of the arterial component (by 45.7-51%) and venous pulsation (improved efflux of blood) with normally inherent values for parameters of tonus of precapillary and postcapillary vessels (V/A, DKI, DSI), as well as by disappearance of the venous wave. All this was indicative of appreciable improvement of blood

circulation conditions in the brain with use of LBNP: sensations of blood rushing to the head and heavy-headedness disappeared or diminished. I. I. Kas'yan et al. [7] also observed improvement of venous efflux from the cranial cavity in all crew members of the Salyut-4 station under the effect of LBNP.

Since improvement of the CDR's well-being, against the background of diminished signs of intracranial plethora during exposure to LBNP, was associated with noticeable elevation of the low parameters of tonus of veins and particularly arterioles of the brain, there are grounds to believe that the marked dilatation of arterioles and small arteries observed in flight could be related to elevated intracranial pressure. Even less marked dilatation of cerebral vessels leads to elevation of intracranial pressure [8]. At the end of the flight, with use of LBNP there was incomplete normalization of cerebral hemodynamics: the venous waves on the REG did not disappear.

On the 1st postflight day, both cosmonauts failed to demonstrate venous waves on the REG (see Figure 1d), i.e., signs of difficult venous efflux from the cranial cavity, which had been periodically seen throughout the flight. As compared to preflight data for the CDR, we found (see Figure 1, 1d, 2a) a decrease in pulsed blood filling of hemispheres by 47% (bottom of normal range) against a background of diminished tonus of vessels, particularly those with a large caliber (α/T% by 40%). The nature of interhemispheric asymmetry of pulsed filling observed preflight ($s>d$) persisted (see Figure 1, 1d). In the FLE, pulsed filling of the hemispheres on the 1st postflight day exceeded the preflight level by 40% (see Figure 1, 2d, 2b) against a background of increased vascular tonus (α/T% by 25% for hemispheric and bimastoid REG's) and some dilatation of arterioles and veins. On the 3d postflight day, the CDR presented some decrease in tonus of arterioles and veins, i.e., active vasodilatation, which was associated with increased filling to normal values and disappearance of interhemisphere asymmetry. In the FLE, asymmetry of filling of hemispheres with blood also leveled off against a background of decline to preflight levels and some decrease in tonus of arterioles and veins.

On the 1st postflight day there was noticeable increase in pulsed filling of the lungs, as compared to the preflight level--by 28.5% for the CDR (Figure 3a) and 49.2% for the FLE (Figure 3b) with concurrent significant increase in tonus of large vessels (α/T% on lung REG rose by 53% for the former and 38.1% for the latter). The rheographic signs of pulmonary vascular hypertension were confirmed by auscultation (accented second tone over the pulmonary artery) and results of echocardiography, which showed signs of overloading of the right heart (according to the data of O. A. At'kov and G. A. Fomina). Such a coincidence of rheographic and echocardiographic data is understandable, since the shape and amplitude of the systolic wave on the lung RG depend on stroke volume of the right ventricle and pulse pressure in the pulmonary artery [9]. The signs of pulmonary hypertension and relative hypervolemia, which were more marked in the FLE (see Figure 3), leveled off by the 12th postflight day.

On the 1st postflight day, the CDR presented 43.8% increase in α/T% on the rheohepatogram, with 130% increase in pulsed delivery of blood to the liver; in the FLE, α/T% increased by 79% without appreciable change in blood filling

of the liver. On the 3d day, the CDR showed a decrease in filling and tonus of large vessels of the liver to the preflight level, i.e., normalization against a background of increased arteriolar tonus.

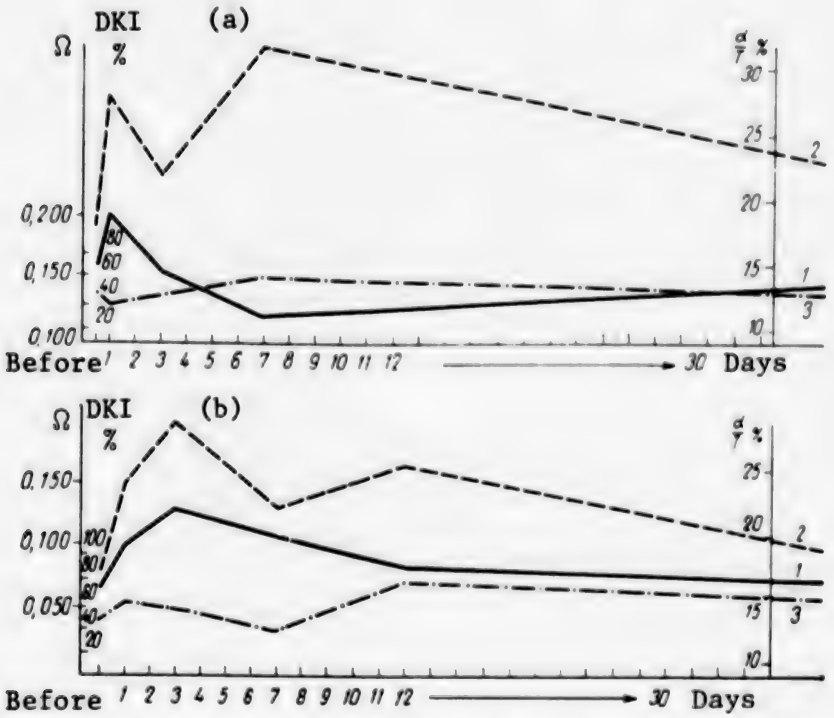

Figure 3. Dynamics of RG parameters of right lung for CDR (a) and FLE (b) before and after flight at rest

1) RG amplitude (in fractions of ohms) 2) α/T% 3) DKI (%)

On the 1st postflight day, the decrease in crural arteriolar tonus (DKI by 32.4%) was associated with significant increase in filling with blood in the CDR, whereas in the FLE a more marked decrease of DKI (by 59.9%) was associated with appreciable decrease in delivery of blood to the lower leg. On the 3d day, the parameters of tonus and blood filling came close to preflight values in the CDR, whereas crural filling was still low in the FLE.

On the 30th postflight day, the parameters of pulsed filling with blood, tonus and elasticity of vessels in all regions examined came close to preflight levels. There was merely asymmetry of tonus of arterioles and veins in the cerebral hemispheres and fingers, i.e., changes in vasomotor regulation. In addition, the tonus of large vessels of the liver was still high, against a background of normalization of delivery of blood to it.

The most marked changes in regional hemodynamic reactions, particularly in the brain, to postural loads were observed in the 1st postflight week. On the 1st postflight day, the orthostatic test on the CDR was associated with

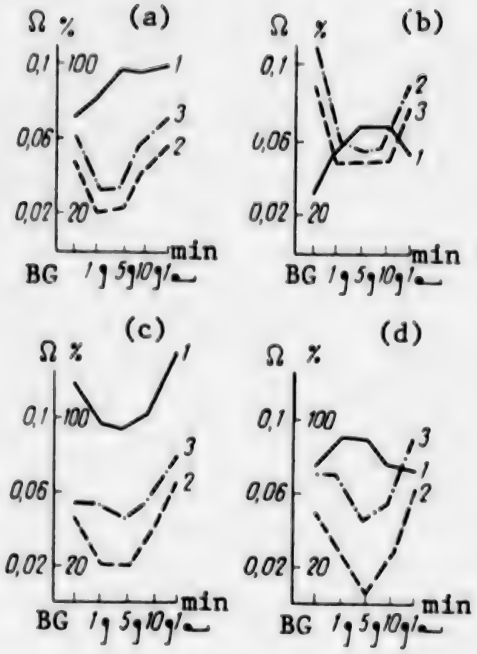

Figure 4.
Dynamics of parameters of REG in frontomastoid (a and c) and bimastoid (b and d) leads in CDR during passive orthostatic test on 1st postflight day
a, b) preflight
c, d) postflight
1) REG amplitude (Ω)
2) DKI (%)
3) DSI (%)
BG) background

significant decline of DKI on the REG of the right hemisphere, from 52.4 to 18.7% (Figure 4c) and on the bimastoid REG from 45% to 0 (Figure 4d); DSI diminished accordingly, from 55 to 44.4% and from 71 to 38%. Pulsed filling of the hemispheres decreased by 50% and that of the vertebrobasilar system by 65% as compared to the background level, with concurrent increase in pulse rate from 72 to 103/min (versus 71 to 85 preflight).

In the FLE, the orthostatic test on the 1st postflight day was associated with increase in pulse rate from 62 to 82/min, decline of DKI on the bimastoid REG from 87.5 to 12.9% (by 86.2%), whereas it had shown virtually no change before the flight. Immediately after the orthostatic test, there was development of marked reactive cerebral hyperemia (delivery of blood to hemispheres increased by 43%, versus only 20% before the flight). As we see, on the 1st postflight day there was considerably less tolerance of the orthostatic test: appearance of signs of marked arterial hypotension, particularly in the vertebrobasilar basin. Yet, before the flight, there was more significant decrease in arteriolar tonus in the system of the internal carotid artery of the CDR (Figure 4a) than in the vertebrobasilar system (see Figure 4b). Consequently, as a result of the flight, the CDR showed a functional change in Willis' circle, the correlation between hemodynamic changes in the two main pools of the brain. While the zone of hemodynamic equilibrium between the carotid and vertebrobasilar systems was shifted in the direction of the former under orthostatic conditions before the flight, which is inherent in young people [3, 12], it shifted in the direction of the latter after the flight. This was also demonstrable on the 5th postflight day--orthostatic tolerance was also diminished in the CDR: DKI on the hemisphere REG diminished from 70.7 to 29.9% and on the bimastoid REG from 78.1% to 0 in the 5th-7th min of the load, i.e., there was marked hypotonia in the vertebrobasilar system, where pulsed filling decreased by 50%, versus 30% in the hemisphere.

In the FLE, some improvement of orthostatic tolerance was manifested by considerably less decrease in tonus of cerebral arterioles (no more than 34%); however, the marked reactive hyperemia of the brain (blood filling increased by 58.9%, as compared to pretest values) and sensation of the head-down tilt at -10°, which appeared immediately after returning to horizontal position,

were indicative of diminished delivery of blood to the brain during the orthostatic test to a critical level, close to ischemic.

On the 13th postflight day, there was significant improvement of orthostatic tolerance and the regional hemodynamic reaction was close to the preflight value.

Head-down tilt on the 1st postflight day was associated, in the CDR, with considerable increase of DKI and DSI on the REG of the hemisphere, with a lesser increment of pulsed filling (70%) than before the flight (160%). In the vertebrobasilar system, arteriolar and venous tonus during the antiorthostatic test decreased less than before the flight, and this was associated with considerably less increase in pulsed filling--by 100% (versus 280% before the flight). In the FLE, the high background values for DKI and DSI (88.9 and 94.5%) showed virtually no change during head-down tilt, and they were associated with less increase in delivery of blood to the hemisphere, by 44%, the increase constituting 110% for the vertebrobasilar system (versus 103 and 180%, respectively, preflight). On the 5th postflight day, antiorthostasis [head-down tilt] of -30°elicited a decline of DKI and DSI in the CDR in both pools, with less increase in pulsed filling than before the flight. In the FLE, during head-down tilt, even at -45°, there was increase in tonus of arterioles and veins, which was associated with less increase in pulsed filling (by only 40-100%). As we see, there was considerable improvement in postflight tolerance of head-down tilt in both cosmonauts.

On the 13th day, these manifestations of the consequences of cardiovascular adaptation to prolonged weightlessness [13] were less marked in both crew members than on the 5th day. As shown above, the restriction of intracranial plethora in the FLE during the flight was obtained chiefly by triggering of the veno-arterial reflex--constriction of precapillaries) and in the CDR, by adaptive dilatation of cerebral vessels, intensification of contractility and active cardiac diastole. As a result of these mechanisms of adaptation to weightlessness, the volume of intracranial blood filling remained stable in weightlessness, and it rarely exceeded the range of physiological fluctuations.

At the same time, the significant decrease in tonus of cerebral arterioles, particularly in the vertebrobasilar system, in the 1st postflight week during orthostatic tests, against the background of marked tachycardia, was indicative of weakening of mechanisms of regulating cerebral circulation as a result of inflight development of cardiovascular system deconditioning [2, 13, 14].

On the 30th postflight day, orthostatic stability was entirely restored, judging by the dynamics of pulse rate and regional RG, in both cosmonauts, while the nature and severity of cerebral hemodynamic changes during the antiorthostatic test were close to preflight findings, which was indicative of readaptation of the cardiovascular system to earth's gravity.

Thus, there was rather rapid recovery of parameters of regional hemodynamics, particularly in the CDR, which was indicative of the functional nature of the changes that had occurred. The differences in restoration of some parameters

of regional hemodynamics in the cosmonauts are apparently attributable to individual distinctions of adaptation to weightlessness, and they are related both to the age-related distinctions of the cardiovascular system and scope of inflight preventive measures and postflight rehabilitation measures.

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FUNCTIONAL CAPACITIES OF ELDERLY SUBJECTS EXPOSED TO SIMULATED SPACEFLIGHT FACTORS

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 18, No 4, Jul-Aug 84 (manuscript received 2 Aug 83) pp 29-32

[Article by T. N. Krupina, Kh. Kh. Yarullin, N. P. Artamonova, V. A. Gornago, D. A. Alekseyev, N. I. Tsyganova, M. P. Kuz'min, L. M. Filatova, L. A. Fotina, O. A. Smirnov and N. D. Vikharev]

[English abstract from source] In response to stimulated space flights cardiovascular and metabolic changes of 86 volunteers, aged 40-49 and 50-56, were similar to those of young people (25-39 years old). In most aged test subjects, the changes produced by by 8-day head-down tilt (-8°) and 7-day water immersion were moderate and reversible. This type of variation of the adaptive-compensatory reactions give evidence that aged people have sufficiently high functional capabilities. Nevertheless, 36% test subjects, aged 40-49, and 50% test subjects, aged 50-56, displayed certain features suggesting a reduction of the adaptive-compensatory capabilities (functional reserves) as a result of age-related and atherosclerotic changes of the cardiovascular system.

[Text] It is known that age-related changes in metabolism, the cardiovascular system (CVS) and different elements of their neurohumoral regulation lead to restriction of the range of adaptability of the body. To date, sufficient data have been accumulated on the effect of ground-based factors simulating weightlessness and actual spaceflights on the CVS and metabolism in young people. At the same time, the CVS and metabolism were studied in older subjects only during actual spaceflights [1, 3, 12]. There is still virtually no integral conception of the distinctions of adaptive and compensatory reactions of the circulatory system and metabolism in older individuals to simulated and real flight conditions. Yet, the incidence of cardiovascular diseases increases with age [2, 4-7, 8, 14]. Consequently, the risk of diseases during spaceflights increases when older individuals (particularly those over 50 years of age) are allowed to fly.

All this is indicative of the great practical importance of this matter. Our objective here was to investigate the distinctions of reactions and adaptive capacities of the CVS and other systems of older individuals in response to simulated spaceflight factors.

Methods

The electrocardiogram was recorded in 12 standard and 3 Neb leads, as well as a continuous dynamic EKG (DEKG) for 24 h in order to study bioelectrical activity of the heart. Dynamics of the heart were assessed from data on phonocardiograms and polycardiograms. To study regional and central hemodynamics, we recorded rheoencephalograms (REG) in the frontomastoid and bimastoid leads, rheograms (RG) of the right lung, liver and leg, stroke volume of the heart (SV) by the impedance method according to Kubicek [13] and the tachooscillogram according to N. N. Savitskiy.

We measured linear velocity of blood flow in the carotid, vertebral and supratrochlear arteries by the method of ultrasonic Doppler sonography with compression tests. We examined external respiration and gas exchange, lipid, carbohydrate, protein and fluid-electrolyte metabolism, the adrenosympathetic, enzymatic and blood-clotting systems.

The age-related distinctions and range of functional capacities of the CVS and metabolism were studied in 86 elderly male subjects under ordinary conditions at rest and with use of such load tests as the passive orthostatic test of +70° for 20 min and head-down tilt at angles of -15 and 30° for 5 min each, lower body negative pressure (LBNP, at -25 mm Hg for 2 min, -35 mm Hg for 3 min, -40 mm Hg and -50 mm Hg for 5 min each), submaximum and maximum physical loads on a bicycle ergometer. The first group (40-49 years old) consisted of 62 men and the second, of 24 subjects 50-56 years of age; 40 healthy men 25-39 years of age served as a control. In addition, we tested reactions and distinctions of compensatory-adaptive mechanisms of the CVS and metabolic changes during simulated weightlessness (8-day head-down tilt at -8° and 7-day immersion by the method of unsupported "dry submersion" [9]) on 22 of the older subjects and 14 from the control group. The techniques for rheography were described previously [10].

Results and Discussion

The results of our studies revealed that the CVS reaction and metabolic changes in response to factors simulating spaceflight conditions presented essentially the same clinical and physiological orientation in both older age groups (judging by the mean values of tested parameters) as young people. However, older subjects (particularly those over 50 years of age) more often presented a tendency toward hypertensive reactions to load tests and a somewhat slower type of restoration of parameters to the background levels.

As compared to young men, the older subjects presented greater orthostatic tolerance in the postural tests and LBNP. At the same time, some of the older subjects, who had signs of moderate atherosclerosis or vegetovascular dystonia of the hypertensive type, had substantially lower tolerance of these tests (particularly after HDT [head-down tilt--antiorthostatic position] and immersion) due to worsening of adaptive capabilities of the CVS and nervous system.

Identical physical work capacity in men of all three age groups, under ordinary conditions and after HDT and immersion, was obtained in the older men due to greater intensity of compensatory and adaptive mechanisms of the body, as

manifested by considerably greater change in heart rate (HR), blood pressure and the EKG, as well as cerebral and central hemodynamics. The appearance of mildly ischemic reactions on the EKG after a physical load in 20% of the older men (mainly those over 50 years of age) is indicative of temporary inconsistency between requirement and delivery of oxygenated blood to the myocardium. These signs of poorer blood supply to the myocardium are attributable to a significant extent to the vascular factor--atherosclerosis [4-6, 14].

It is equally important that all of the subjects over 50 years of age had a heart rate of 160/min during maximum physical load and half of them, 180/min, i.e., it was above the normal WHO values for this age. For this reason, one should have a more cautious attitude toward WHO recommendations when making indirect determination of maximum oxygen uptake according to the standard age-related values for maximum pulse rate.

As compared to subjects 40-49 years of age, in men 50 to 56 years old the decline of functional reserves of the body after hypokinesia was also more marked.

Examination of central and regional hemodynamics in most older subjects under ordinary conditions revealed some (within the range of the norm for their age) decrease in stroke and minute volumes, pulsed filling of the brain and an overt tendency (particularly after 45 years of age) toward increase in tonus of cerebral arterioles and veins (i.e., vascular resistance) and decreased elasticity of vessels. The relative duration of the anacrotic phase (α/T, %), dicrotic (DKI) and diastolic (DSI) indexes of the REG turned out to be the most informative for objective evaluation of elasticity (rigidity) of tonus and reactivity of vessels in determining the extent of age-related and atherosclerotic changes [11].

With use of HDT, the changes in the vascular system of the brain and eye, as well as in intraocular pressure, in most older subjects were similar to those in young people, which is indicative of adequate compensatory capacities of cerebral circulation and the eye.

With use of load tests and simulated weightlessness (HDT, immersion), the metaboiic changes were generally in the same direction in older men as in young subjects. With age, there was some elevation of blood cholesterol, β-lipoprotein and triglyceride levels, as well as appearance of atherogenic types of lipids.

The change in blood coagulation under ordinary conditions, during load tests, as well as HDT and immersion, were indicative of adequate compensatory capacities in older subjects. However, during immersion, hypercoagulation was associated with less rise in blood fibrinolytic activity, which is indicative of the possibility of thrombolytic complications under such conditions.

In most of the tested older subjects, the functional changes in the systems examined during 8-day HDT at -8° and 7-day immersion were quite moderate and reversible. Such dynamics of adaptive and compensatory reactions indicate that older individuals (40-56 years) have a sufficient level of functional capacities and can be allowed to participate in the Soviet space program.

At the same time, a number of distinctions referable to health status, which were indicative of overt decline of adaptive capacities (functional reserves of the body) as a result of age-related and atherosclerotic changes in the CVS, were demonstrated in 36% of the subjects 40-49 years of age and 50% of those over 50 years old. The significant changes we demonstrated in lipid metabolism of these subjects, with appearance of atherogenic types, were associated with moderate atherosclerosis of cerebral and retinal vessels, diminished pulsed filling of cerebral hemispheres and blood flow in the carotid or vertebral arteries, poorer function of communicating arteries of Willis' circle and increase in thrombogenic potential of blood.

The chosen set of clinical-instrumentation and biochemical tests, as well as the set of functional loads on the CVS, turned out to be quite informative for evaluation of the condition of this system and metabolism in older subjects under both ordinary conditions and during week-long HDT and immersion.

Analysis of data referable to well-being of the subjects, parameters of CVS function, lipid metabolism clotting system and fibrinolytic activity of blood under ordinary conditions and their dynamics during load tests, week-long HDT of -8° and immersion enabled us to offer an expert characterization of the distinctions of favorable and unfavorable states in older individuals and to define criteria for evaluating them.

1. Favorable distinctions of health status of older individuals are as follows:

a) Under ordinary conditions--total cholesterol up to 200 mg%, β-lipoproteins up to 650 mg%, their cholesterol content to 90 mg% and blood serum triglycerides to 160 mg%; normotonic, mildly hypertonic (DKI up to 70%) and atherosclerotic (α/T, % to 22%) types of REG; good or satisfactory function of communicating arteries of Willis' circle and minor decline of linear velocity of blood flow in carotid and vertebral arteries according to Doppler sonography data; tendency of α rhythm on EEG toward desynchronization or hypersynchronization (amplitude of basic rhythm up to 110 μV); presence on EEG of polymorphic slow activity with amplitude up to 50 μV and index up to 15%; presence on EEG of low-frequency (up to 20 cycles/s) β-activity with amplitude up to 10 μV in leads from frontal regions of the brain.

b) During load tests--normotonic or mild hypertonic type of CVS reactions to load tests without changes in ST segment on the EKG, or with no more than 1 mm depression; normotonic or moderately hypertonic type of reactions of cerebral, pulmonary and peripheral circulation with moderate decrease in pulsed blood filling of the brain and lungs, as well as mild increase in filling of crural veins in orthostatic position and during LBNP; appearance of driving response (adoption of rhythm in low-frequency range) on EEG in test with rhythmic photic stimulation; absence of paroxysmal activity in test with hyperventilation of the lungs (5 min); moderate hypercoagulation with adequate intensification of blood fibrinolytic activity.

2. Unfavorable expert criteria for assessing health status of older individuals are as follows:

a) Under ordinary conditions--high levels of lipoproteins (over 850 mg%), triglycerides in blood serum (over 250 mg%), cholesterol and lipoproteins (over 120 mg%); marked atherosclerotic (α/T,% 22-27%) and hypertonic (DKI over 80%), as well as hypotonic (DKI under 30%) types of REG, significant decrease in pulsed filling; significant decrease in blood flow in carotid or vertebral arteries, or their functional insufficiency according to results of Doppler sonography, nonfunctional arteries of Willis' circle (anterior or both posterior communicating); marked amplitude and frequency disorganization of α rhythm (presence of frequent isolated α waves and short groups of them, "splitting" or "tapering" of α waves, irregular rhythm of 8 to 12-13 cycles/s) or hypersynchronous type of EEG (amplitude of basic rhythm over 110 μV, smoothing of zonal differences); presence on EEG of diffuse polymorphic, particularly focal, slow activity with amplitude in excess of 50 μV and index over 15%; presence on EEG of low-frequency (up to 20 c/s) diffuse β-activity with amplitude in excess of 10 μV; presence in subjects of combination of 2-3 risk factors, such as obesity, impaired lipid metabolism and tendency toward hypertensive reactions, or combination of heavy and long-term smoking with tendency toward hypertensive reactions.

b) During load tests--hypotonic and marked hypertonic types of CVS reactions, ischemic type of reactions according to EKG data (over 1 mm depression of ST segment); marked tachycardia, which changes to bradycardia with development of a collaptoid state, appearance of frequent extrasystole of heterotopic rhythms; considerable decrease in circulation minute volume; very marked hypotonic (decline of DKI by more than 40% of base value) and hypertonic (DKI over 90%) types of REG changes in response to orthostatic or LBNP tests, significant (over 40% of base value) decrease in pulsed filling of the brain (followed by its drastic increase when a collaptoid state develops), drastic increase in delivery of blood to crural veins; drastic (by more than 200%, as compared to background level) in pulsed filling of the brain and appearance of a pronounced venous wave on the REG with -30° HDT; tendency toward increased clotting activity of blood with concurrent slowing of fibrinolysis; appearance of paroxysmal activity on the EEG during test with 5-min hyperventilation of the lungs.

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EFFECT OF FLUID AND SALT SUPPLEMENTS TO FOOD ALLOWANCE ON ENDURANCE OF HEAD-TO-PELVIS ACCELERATIONS FOLLOWING 7-DAY 'DRY' IMMERSION AND UNDER ORDINARY MOTOR ACTIVITY CONDITIONS

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 18, No 4, Jul-Aug 84 (manuscript received 24 May 83) pp 33-37

[Article by N. I. Kokova]

[English abstract from source] The effect of water-salt supplements as an agent increasing human tolerance to head-to-feet acceleration with a slow onset was examined. The test subjects were rotated in a 7.25 m arm centrifuge after 7-day dry immersion or normal motor activity. The water-salt supplements were given at a dose of 0.15 g NaCl and 18 mℓ water per kg body weight (with the total daily dose consumed in four fractions). During immersion fluid retention was significantly higher than during normal activity (818±139.7 mℓ versus 478±69 mℓ). Water-salt supplements consumed produced a positive effect on tolerance to head-to-feet acceleration. During centrifugation after water-salt supplementation the physiological responses were less strained. Water-salt supplements taken on the last immersion day increased the tolerance level as compared to the control. The amount of the fluid retained in the body was found to be inversely proportional to the tolerance level. It is concluded that water-salt supplements may be recommended to increase tolerance to head-to-feet acceleration in aerospace medicine.

[Text] Dehydration, which is consistently observed during spaceflights and under conditions simulating weightlessness, is viewed as one of the important causes of man's diminished resistance to gravity factors due to redistribution of blood to the lower extremities [5, 8]. For this reason, artificial increase in hydration of the human body is a pathogenetically justified preventive means of enhancing his resistance to orthostatic factors and longitudinally oriented head-to-feet acceleration with both the normal level of hydration and, particularly, hypohydration. The increase in circulating blood volume under such conditions should lead to adequate return of venous blood to the heart and thus increase stroke volume and cardiac output. It was found that rehydration by means of intake of additional amounts of fluid is particularly effective in preventing orthostatic disturbances [1-3]. Encouraging results, although not as convincing, were obtained with rehydration of the body in

the case of endurance of head-to-feet accelerations [6, 7]. In assessing the potential value of this method, much significance is attributed to the method of rehydration, as well as such characteristics of gravity factors as magnitude, duration and rate of build-up of accelerations. If successful, one could expect that the armamentarium referable to means of protection against accelerations will be enriched with a new element, which is consistent with the objective demands of aviation and space medicine.

Our objective here was to assess the possibility of enhancing tolerance of head-pelvis accelerations that are built up slowly by means of water and salt supplements to the diet under conditions of 7-day simulation of weightlessness by the method of "dry" immersion [4].

Methods

Three series of studies were conducted with the partipation of 18 male subjects 28-32 years of age (6 men in each series). All of the subjects had previously experienced rotation on a centrifuge. In each series, they were submitted to head-pelvis accelerations three times (at 5-7-day intervals), with a build-up gradient of 0.003 G/s on a centrifuge with 7.25 m arm. The first two rotations in each series served as a control.

In the first two series, weightlessness was simulated by 7-day "dry" immersion between the second and third rotations on the centrifuge, whereas in the third series the subjects continued on their usual routines. No preventive agents were used to enhance man's resistance to accelerations in the first series of tests. The subjects involved in the second and third series were given a water and salt supplement to their diet prior to the last rotation on the centrifuge [3]. In the second series, the water and salt supplement was started on the 7th day of immersion and in the third series, under conditions of ordinary motor activity. Each man was given 0.15 g sodium chloride and 18 mℓ water/kg weight, in 3 divided doses, 16, 12 and 2 h before rotation. In addition, the subjects took 200-300 mℓ fluid in the form of tea 40 min before exposure to accelerations.

Thus, the first series served as a control and was intended to demonstrate the degree of decline in tolerance of accelerations under the effect of immersion. In the second series, we assessed the efficacy of rehydration and in the third, that of relative hyperhydration, since the water and salt supplements were combined in this instance with initial fluid saturation.

The subject's request to stop rotation, visual disturbances, diminished amplitude of pulse in vessels of the earlobe to the isoline, systolic blood pressure drop in vessels of the earlobe to less than 40 mm Hg, sinus tachycardia in excess of 180/min or marked disturbances of cardiac rhythm were the criteria of the limit of endurance of accelerations. In the third series of tests, unlike the first two, exposure to accelerations was limited to 3 G, since this was found to be sufficient to obtain data about physiological reactions to accelerations that were comparable to the preceding series.

In all of the studies, we recorded the EKG in the three Neb leads, heart rate, systolic blood pressure in earlobe vessels and pressure in the brachial artery

by the Korotkov method. In addition, in the third series we measured cardiac stroke volume using thoracic tetrapolar rheography. The time and nature of appearance of visual disorders were determined on the basis of the subjects' reports. During the period of intake of the water and salt supplement to the diet, balance studies of fluid intake and fluid output were made to evaluate fluid retention prior to exposure to accelerations.

All of the data were processed by the method of variation statistics according to Student ($P<0.05$).

Results and Discussion

As a rule, there was increase in tolerance of head-feet accelerations in the first and second series of subjects during the second control rotation on the centrifuge (probably due to a conditioning effect), and it constituted 4.77±0.15 units in the first series, 4.6±0.14 units in the second (Figure 1). We took the results of the second rotation on the centrifuge as the parameter of initial endurance in order to obtain more reliable data. Seven-day "dry" immersion diminished, with statistical reliability, endurance of accelerations in both series. In the first, control series, this decline was more marked (to 3.63±0.17 units, i.e., by a mean of 1.143±0.187 units) than in the second one, where fluid and salt supplements to the diet were used (where tolerance decreased to 3.85±0.06 units, i.e., the difference averaged 0.766±0.123 units). Moreover, in the first series, only half the subjects were able to tolerate accelerations in excess of 3.5 units, whereas in the second series all of the subjects were up to this task.

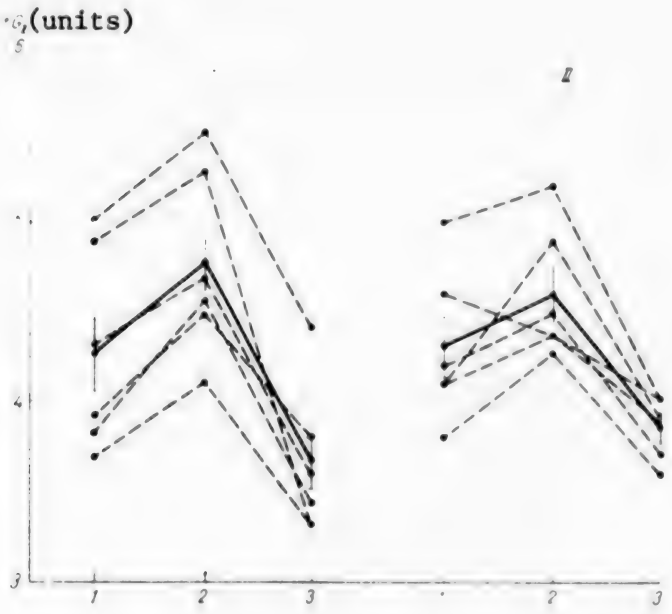

Figure 1. Tolerance of head-feet accelerations in subjects used in the first and second series of tests with rotation on centrifuge before (1 and 2) and after (3) "dry" immersion. Dash lines--individual parameters, solid line--group mean

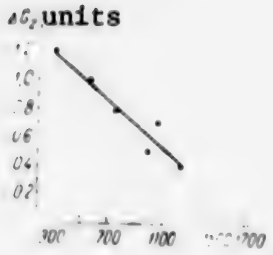

Figure 2.
Individual decline of tolerance of head-feet (ΔG_z) accelerations after immersion as a function of amount of fluid retained in the body.
X-axis, fluid retention (mℓ)

The subjective reports indicated that rotation after immersion in the second series, in which water and salt supplements were used, was tolerated somewhat better than analogous rotation in the first series, where water and salt supplements were not used. The subjects were less tired at the end of exposure to accelerations, and it was somewhat easier for them to retain muscular tension.

During rotation after immersion, marked visual disorders were recorded in 50% of the cases in the first control series. After intake of the fluid-salt supplement, overt visual disorder lasting 15 s in the form of "gray film" was noted during rotation in only one subject. His "gray film" coincided with decrease in amplitude of vascular pulse of the earlobe to the isoline. It was found that this subject retained the least fluid, as compared to the rest of the subjects, and retention constituted only 310 mℓ. After use of water and salt supplements, one more subject had a visual disorder during rotation, but it was brief and mild. According to the subject's report, he observed only "some change in contrast of objects." After increasing muscle tension his vision recovered rapidly.

Table 1. Dynamics of parameters of cardiovascular system (M±m) with exposure to head-feet accelerations up to 3 G following immersion without use of preventive measures (I) and with water and salt supplement to food allowance (II)

Parameter	Series	Background	Accelerations up to 3 G
Heart rate, per min	I	80±4,57	169±6,49
	II	85±5,48	156±7,29
Pulsed arterial pressure in brachial region, mm Hg	I	56±3,53	59±6,57
	II	56±3,97	77±12,24
Systolic blood pressure in earlobe vessel, mm Hg	I	117±4,03	90±13,3
	II	127,±5,44	88±6,09

A comparison of physiological reactions to accelerations in these two series, illustrated in Table 1, shows that it was necessary to limit accelerations to 3 U, since higher levels could not, as we have already noted, be tolerated by all of the subjects. We were impressed by the fact that the responses of heart rate and particularly pulsed arterial pressure were obviously preferable in the second series.

Thus, the water and salt supplements to the diet, which were used at the end of immersion, had a beneficial effect on tolerance of accelerations in the

head-to-feet direction and attenuated the physiological changes associated with comparable accelerations.

It was of definite interest to determine whether the water and salt supplements would be beneficial or, at least harmless if used against the background of a water level in the body that was not altered by any special measures.

This question was studied in the third series of tests, where we repeated three-fold exposure to accelerations (Table 2) sufficient to make comparisons with the preceding series. The test methods in this series additionally included measurement of stroke volume.

Table 2. Dynamics of parameters of cardiovascular system (M±m) with exposure to head-to-feet accelerations up to 3 G during normal motor activity (group 1) and after use of water and salt supplements to diet (group 2)

Parameter	Group	Background	To 3 G
Heart rate, per min	1	90±4,66	169±7,98
	2	85±6,69	161±7,84
Systolic pressure in earlobe vessels, mm Hg	1	98±5,76	100±9,1
	2	98±4,79	118±6,56*
Stroke volume, mℓ	1	117±21,7	66±6,4
	2	105±19,77	105±10,38**

* $P<0,05$
** $P<0,01$.

We failed to demonstrate any adverse consequences after use of water and salt supplements. We found that tolerance of accelerations improved, according to the demonstrated physiological reactions, after intake of water and salt supplements. This was indicated by the less marked intensity of function of physiological systems during exposure to accelerations after use of water and salt supplements, as compared to control rotation. With accelerations of 3 units, there was less marked sinus tachycardia, blood pressure in earlobe vessels was 18% higher and stroke volume was 37% higher than the control value (see Table 2). After use of water and salt supplements, stroke volume of the heart remained stable throughout the period of rotation, whereas in the control this parameter dropped to 47% of the control value at 3 G accelerations. There is every reason to relate this effect to retention of excessive fluid which, according to the results of the balance studies, constituted 478±69 mℓ at the time of rotation. The analogous parameter was higher in the second series, constituting 818±139.7 mℓ. And this is understandable, since the water and salt supplement was used here against a background of partial dehydration due to simulated weightlessness. The question arises as to why tolerance of accelerations still diminished in the presence of relatively greater fluid retention. Of course, there are several other mechanisms, which we do not discuss here, in addition to dehydration that are related to the origin of antigravity functional disorders. Still, it is interesting to follow through on the specific data of the second series with regard to individual decline of tolerance of head-feet accelerations after immersion as

a function of amount of fluid retained in the body. Such a comparison is made in Figure 2. This figure shows that the amount of fluid retained differs and ranges from 310 mℓ to 1230 mℓ. Minimal decline of tolerance of accelerations (by 0.4 units) after immersion was referable to the subject whose fluid retention constituted 1230 mℓ, i.e., the largest amount. Conversely, the subject who presented the least fluid retention (310 mℓ) demonstrated the most significant decline of tolerance of accelerations after immersion, by 1.2 units. He also presented a persistant and obvious visual disorder in the form of a "gray film" during rotation. Probably we cannot discuss a beneficial effect of the water and salt supplement in this case.

On the whole, we found a close inverse correlation between amount of fluid retained and extent of decline in tolerance of accelerations, and a regression function that can be described by the following equation:

$$y = 1.45 - 0.00084 \cdot x$$

where y is the decline in tolerance of head-feet accelerations following immersion, as compared to control rotation on the centrifuge (units) and x is the volume of fluid retained (in mℓ). The coefficient of correlation is $r = -0.96 \pm 0.03$. If we continue the line of regression to its intersection with the x-axis, we can assume that, under these specific test conditions, it is necessary to retain approximately 1700 mℓ fluid in order to prevent a decline in tolerance of head-to-feet accelerations following immersion.

Thus, the results of these studies revealed that an increase in hydration of the body by means of water and salt supplements to the food allowance has a beneficial effect on tolerance of head-to-feet accelerations. During rotation after use of the water and salt supplement, in the case of prior normal motor activity, there was less marked intensity of function of physiological systems than in the control studies: less sinus tachycardia, higher blood pressure in earlobe vessels, while stroke volume was considerably greater (by 37%) than the control value, and it remained stable throughout the period of rotation. Intake of water and salt supplements on the last day of immersion enhanced man's tolerance of head-to-feet accelerations, as compared to control findings. We found a close inverse relationship between amount of fluid retained and magnitude of decline in tolerance of accelerations.

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FREQUENCY AND NATURE OF ELECTROCARDIOGRAPHIC DISTURBANCES IN DOGS DURING SINGLE AND REPEATED EXPOSURE TO $+G_z$ ACCELERATIONS

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 18, No 4, Jul-Aug 84 (manuscript received 31 Mar 83) pp 37-41

[Article by R. A. Vartbaronov, G. D. Glod, N. N. Uglova, M. N. Khomenko and I. S. Rolik]

[English abstract from source] Pathological changes of the ECG were examined in 10 adult dogs exposed to $+G_z$ once a day or 3 times a week for three days a week during 2 to 12 weeks. In response to acceleration all of the dogs developed ECG changes. The frequency and level of these disorders were dependent on the acceleration magnitude and the health state that varied during repeated exposure. These findings were used to develop a 5-score scale for measuring ECG disorders and to identify phase changes in acceleration tolerance during repeated exposure to $+G_z$. It was also demonstrated that animals can be specifically trained to tolerate sustained and high acceleration $+G_z$.

[Text] Numerous studies have established that the cardiovascular system plays the leading part in the body's adaptation to single and repeated exposure to head-pelvis accelerations ($+G_z$). However, there are only isolated works dealing with the frequency and nature of EKG disturbances with exposure to $+G_z$ accelerations [2, 4, 5, 7, 9-13].

Thus, Parkhurst et al. observed isolated and multiple extrasystoles, atrioventricular blocks, distortion of QRS complex, disappearance of T wave, respiratory and sinus arrhythmia in 8 out of 14 pilots exposed to +7.5-+9.0 G_z. Analogous EKG disturbances under the effect of $+G_z$ accelerations were also demonstrated by other authors in studies involving man [4, 5, 9, 11, 12], as well as animal experiments [2, 7, 13].

Some researchers do not consider it possible to use rhythm disturbances of the heart (RDH) as a criterion to assess resistance to accelerations [9, 12], while others recommend that they merely be used as an additional criterion [4, 5].

At the same time, it can be assumed on the basis of some studies [10, 13] that the incidence of EKG disturbances will increase with exposure to head-to-feet accelerations of greater magnitude and duration, which increases the urgency of research in this direction.

Methods

We studied the quantitative severity of EKG disturbances as a function of magnitude of accelerations and functional state of the body in order to validate the possibility of using these factors as a criterion of animal tolerance of single and regularly repeated exposures to $+G_z$ accelerations.

Experiments with use of $+G_z$ accelerations were performed on 10 sexually mature intact dogs weighing 6-14 kg. The methods of immobilizing animals on the centrifuge, their preparation for a long-term experiment and program of regularly repeated exposure to $+G_z$ accelerations were described in our preceding work [1]. We determined the maximum tolerance of single exposure to accelerations before the start of a long-term experiment and 1.5-2 months after it, using the method of P. M. Suvorov [4] modified for animals; intervals between exposures constituted 2 min, accelerations were increased from 2 to 20 units, until there was appearance of marked EKG disturbances in the form of group, polytopic extrasystole, recurrent block or migration of pacemaker, block of branches of His' bundle and drastic decrease in ST interval, etc. (see Table). The long-term experiment consisted of three series of tests. In the first and second series, which lasted 0.5 and 3 months, respectively, we used a program of extreme exposure to regularly recurring accelerations [1]. In the third series, which lasted 2 months, we used a conditioning program of exposure; accelerations constituted 2 units on the 1st day of the main experiment, and they were increased by 1 unit on each subsequent experimental day until there was appearance of marked pathological EKG disturbances. Then, 1 month later, we used the principle of extreme exposure to accelerations, as in the first two series.

Evaluation of severity of EKG disturbances in dogs exposed to $+G_z$ accelerations

Score	Severity of EKG disturbances	Extrasystole			Impulse block (His' bundle)			Pacemaker migration		Asystole	
		isolated	recurrent	groups, polytopic	isolated	recurrent	multiple	isolated	recurrent	brief, to 1.5 s	Prolonged, >1.5 s
0	None										
1	Insignificant										
2	Moderate										
3	Marked										
4	Maximum										

Note: Maximum EKG impairment (score 4) differs from marked disturbances (score 3) in that there is concurrent combination of several types of disturbances that progress, leading to the necessity of premature termination of single or repeated exposure to $+G_z$.

The overall load on each animal with repeated exposures was assessed by the index of Burton [6], which is the product of magnitude of acceleration multiplied by exposure time (units × min).

Results and Discussion

The results of our studies revealed that the nature of EKG disturbances related to single and regular exposure to $+G_z$ accelerations does not differ from that described in the literature [2, 4, 5, 7, 9-13]. In order to demonstrate the quantitative functions, we first analyzed the frequency of pathological EKG disturbances with exposure to acceleration one time (Figure 1). As can be seen in Figure 1 (a, b), the incidence of EKG disturbances, including marked ones, is a linear function of magnitude of accelerations. This function was demonstrated both in the background examination of the animals (R = 0.99) and 2 months after regular exposures (R = 0.96). But in the latter instance, approximately the same percentage of EKG disturbances occurred with greater (by 3 G) accelerations as in the background period. This is apparently indicative of increased resistance of animals to single exposure, against the background of regular, repeated exposure. Analysis of these findings (see Figure 1c) shows that, in a certain percentage of cases with use of maximum accelerations, marked EKG disturbances precede by 1-4 units appearance of moderate, gradually progressing disturbances, which is indicative of their appreciable significance to evaluation of functional state of the animals when exposed to $+G_z$ accelerations and, in particular, to prediction of maximum tolerance.

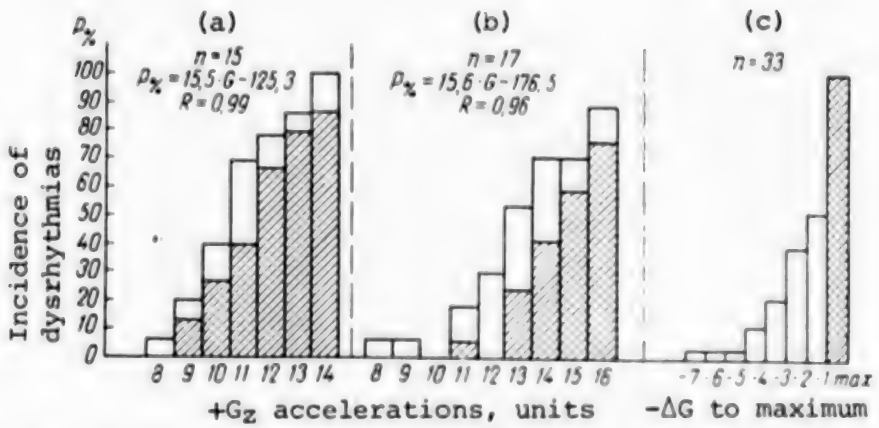

Figure 1. Incidence of disturbances referable to cardiac rhythm in dogs with exposure to accelerations one time. Incidence of such disturbances (P%) as a regression function of accelerations (G) and coefficients of correlation (R) are shown in diagrams (a) and (b)

a) background study
b) 1.5-2 months after regular exposure to $+G_z$
c) upon reaching maximum tolerance
n) number of cases at each magnitude of acceleration

White bars refer to isolated disturbances and cross-hatched, to marked ones. In all instances, relative number of "plateaus" (%) with EKG disturbances is shown.

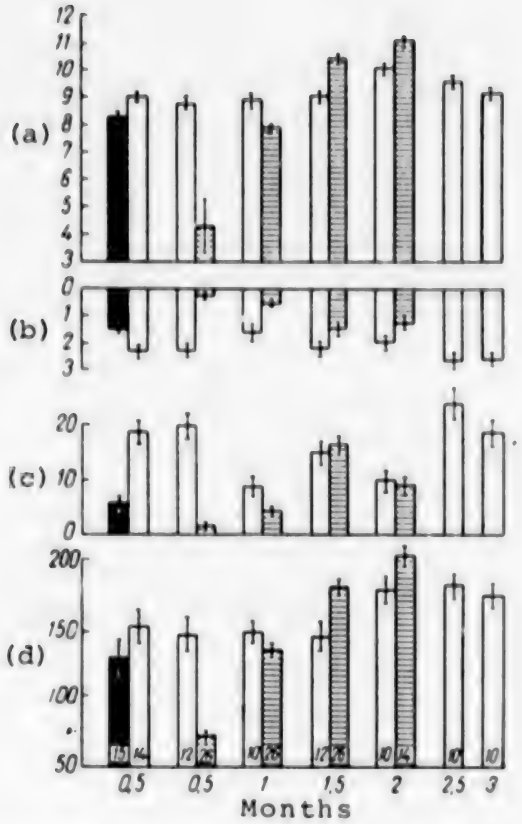

Figure 2.
Incidence of dysrhythmia in dogs during regular exposure to $+G_z$ accelerations for 0.5 to 3 months
Black bars--1st series, white--2d and striped--3d
At bottom of bars in (d), the number of experimental days is given. Number of cases in (a), (b) and (c) is 3 times greater than indicated in (d)
a) maximum $+G_z$ (units)
b) rating of severity of dysrhythmia (point score)
c) incidnece of dysrhythmia (%)
d) overall burden (G × min)

The appearance of marked EKG disturbances as a function of magnitude of accelerations and functional state, as well as relative prognostic value of moderate disturbances, are indicative of the possibility of using marked EKG disturbances as a criterion of canine tolerance of $+G_z$ accelerations, as well as the desirability of making a differentiated qualitative assessment of these disturbances, which is convenient for statistical analysis. For this purpose, we elaborated a scoring system for EKG disturbances in dogs to apply to exposure to $+G_z$ accelerations (see Table).

The classifications of EKG disturbances in electrocardiology [3, 8], which are used in clinical practice, turned out to be unsuitable for our conditions.

The point rating (see Table) was used at the next stage in the study of effect of regular and repeated exposure to accelerations for up to 3 months.

Mean data for half-month (6-7 experimental days) periods of exposure were submitted to statistical analysis. The intensity of repeated exposure was assessed by the magnitude of maximum acceleration generated in each series of single exposure to $+G_z$ and according to Burton's index.

The results are illustrated in Figure 2, which shows that, within a half-month period of exposure to repeated accelerations of submaximum magnitude (1st and 2d series), the severity of EKG disturbances in dogs was related to intensity of the factor, as in the case of single exposure.

Subsequently, as the duration of the experiment increased, the degree of deviations was determined chiefly by the animal's functional state. Thus, while the intensity of the burden in the 2d series (white bars) persisted at the same level after 0.5, 1 and 1.5 months of repeated exposure, the incidence of marked EKG disturbances (% of appearance on different "plateaus") dropped to less than one-half and the score, to two-thirds. Considering the findings at the first stage of this study, it can be assumed that, in this case, there was elevation of functional level of the body. Thereafter, this process progressed, since

there was another decline in incidence of EKG disturbances after 2 months, with increase in intensity of exposure. However, after 3 months of the chronic experiment, with accelerations on the same level as at 2 months, there was a reliable increase in incidence of marked EKG disturbances, which most probably reflected a relative decline of functional state of the body to about the base level in response to regular exposure to $+G_z$. It can be assumed that the fluctuations of severity of EKG disturbances in the course of the long-term experiment reflected the phasic nature of animal tolerance of repeated accelerations.

In the 3d series of studies (bars with horizontal lines), we used a more sparing (conditioning) program of regular exposure to $+G_z$ accelerations.

In this series, in the course of 1.5-month testing, the severity of EKG disturbances gradually increased with increase in intensity of accelerations. By the end of the 2d month of the basic experiment, against a background of increased intensity of exposure, we observed a relative decline in incidence of EKG disturbances and, accordingly, in the rating. A comparison of the results (see Figure 2) of the 2d and 3d series of studies in the 2d month of the basic experiment, when accelerations were extreme in both instances, revealed more marked adaptive changes in the 3d group of dogs. Although the dynamics of cardiovascular reactions were not followed up for a longer time, it can be assumed that a more marked conditioning effect was obtained at this stage due to gradual increase in intensity of accelerations.

Thus, our data confirm the possibility of using marked EKG disturbances in experiments with dogs as a criterion of tolerance of single and repeated exposure to $+G_z$ accelerations, and they are indicative of the desirability of using a point scoring system of evaluating their severity.

On the other hand, the results indicate that the incidence of EKG disturbances with regular exposure to repeated high $+G_z$ accelerations in dogs is phasic in nature, apparently reflecting the phasic changes in the animals' functional state and, perhaps, in their tolerance of repeated exposure to accelerations.

In conclusion, it should be stressed that the theoretical possibility of conditioning for regular exposure to head-feet accelerations was demonstrated on the basis of our findings, such exposure results in a decreased probability of EKG disturbances with the same levels of accelerations.

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EFFECT OF SPACEFLIGHT FACTORS ON RAT BONE MARROW CELLS

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 18, No 4, Jul-Aug 84 (manuscript received 22 Nov 82) pp 41-43

[Article by D. K. Benova, A. K. Bairakova, I. A. Baev and H. G. Nikolov (People's Republic of Bulgaria)]

[English abstract from source] The effect of spaceflight factors, weightlessness in particular, on the genetic structures of bone marrow cells of rats flown for 18.5 days on Cosmos-1129 was investigated. Chromosome aberrations were measured on postflight days 6 and 25. The frequency of unstable chromosome aberrations was similar in the flight, synchronous and vivarium rats. Karyotyping of metaphase plates revealed chromosome aberrations in the flight and synchronous rats. Exposure to weightlessness did not influence the mutagenic effect in bone marrow cells of the rats.

[Text] With man's penetration into space, space biology was confronted with a number of problems related to the mutagenic effect of spaceflight factors. For example, we need an aswer to the question of whether a stay in space could cause damage to the cell's genetic system. The few studies pursued in this direction yielded contradictory results. For example, an increased incidence of chromosome aberrations in lymphocytes was observed postflight in American astronauts [3, 4], whereas no such effect was demonstrated in Soviet cosmonauts [1]. Genetic changes have been found in bacteria and animals [2, 3].

We have made an attempt here to determine whether spaceflight factors influence the mutagenic effect. For this purpose, we made a cytogenetic analysis of rat bone marrow cells.

Methods

Sexually mature Wistar SPF male rats were divided into 3 groups: the 1st consisted of 17 animals flown in space for 18.5 days, the 2d of 18 animals used in a synchronous experiment and the 3d of 21 animals kept in the vivarium. Bone marrow was taken from the femur 6 to 12 h after landing, and on the 6th and 25th postflight days. Specimens for cytological analysis were prepared using our modification of the method described in [6]. Test tubes with collected material were stored for an average of 24-30 h in a refrigerator,

after which they were kept at room temperature (22-24°C) for 2 h. The material was centrifuged, treated with 0.075 M KCl and fixed in acetic acid with methanol in a 1:3 proportion. The preparations were stained with basic fuchsin. We counted unstable chromosome aberrations of the chromosome and chromatid types using a microscope at 12.5×100/×1.35 magnification: chromosome fragments, interstitial deletions, acentric and centric rings, dicentrics and polycentrics, chromatid fragments. Chromosomes from 194 metaphase plates referable to 3 groups were karyotyped on the 6th and 25th postlanding days. Occasionally, we identified only the first 4-5 pairs of chromosomes. The karyotypes were classified according to the instructions of the Committee for Standardization of Rat Karyotypes [4]. We took into consideration stable chromosome aberrations: symmetrical translocations, inversions and deletions. We measured chromosome length in all metaphases.

Results and Discussion

We failed to demonstrate metaphase plates in the bone marrow of 1st to 3d groups of animals 6-12 h after landing. This could probably be attributed to death of many cells due to their long-term (up to 50 h) storage for technical reasons that did not depend on us.

The Table lists the results of cytogenetic analysis of bone marrow cells of animals sacrificed on the 6th postflight day.

Chromosome aberrations in rat bone marrow cells on 6th day after flight aboard Cosmos-1129 biosatellite

Animal group	Number of rats	Number of cells analyzed	Cells with aberrations (M±m), %			Aberrations per cell
			total	chromosome fragments	chromatid fragments	
1	5	292	3,1 ± 1,0	2,1 ± 0,9	0,3 ± 0,3	0,03
2	6	561	3,9 ± 1,0	3,7 ± 1,7	0	0,04
3	5	270	5,1 ± 1,8	4,7 ± 1,7	0,4 ± 0,1	0,06

No differences were demonstrated between parameters of animals in the three groups studied.

It is known that bone marrow is a dividing, asynchronous population of cells. In spite of the fact that some authors reported slower division of its cells after landing, it is quite likely that the chromosome aberrations induced during the flight were eliminated during mitotic divisions in the recovery period up to the 6th day. We found 3 translocations. We discovered 1 translocation in only 1 rat sacrificed on the 25th day, out of 81 karyotyped metaphases of animals in the 1st group. All 72 karyotyped metaphases of animals in the 2d group revealed 2 translocations in 2 rats also sacrificed on the 6th postflight day (see Table). Figures 1 and 2 illustrate a metaphase plate and karyotyped chromosomes of an animal in the 2d group, in which a translocation was demonstrated.

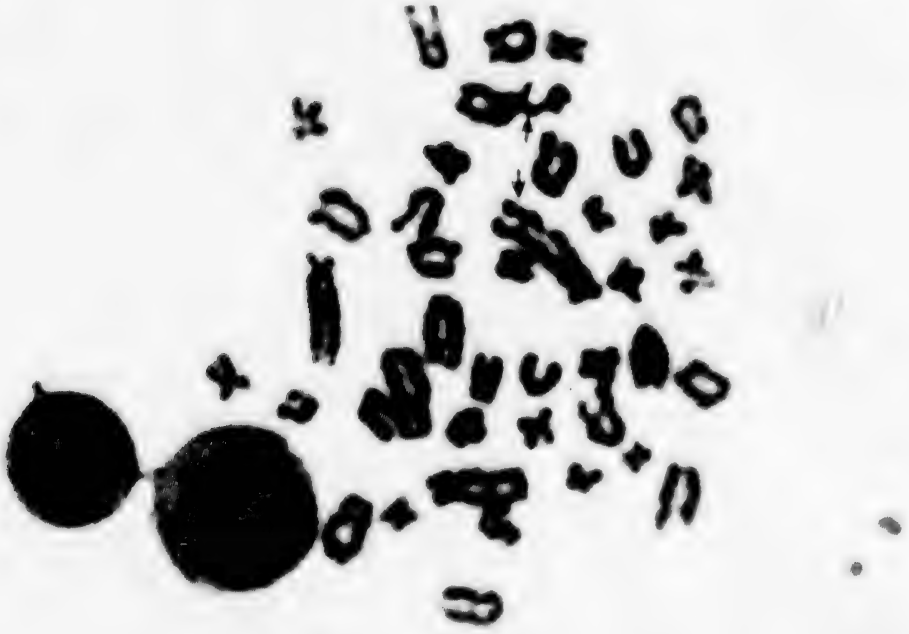

Figure 1. Rat metaphase plate with translocated chromosome (arrowheads)

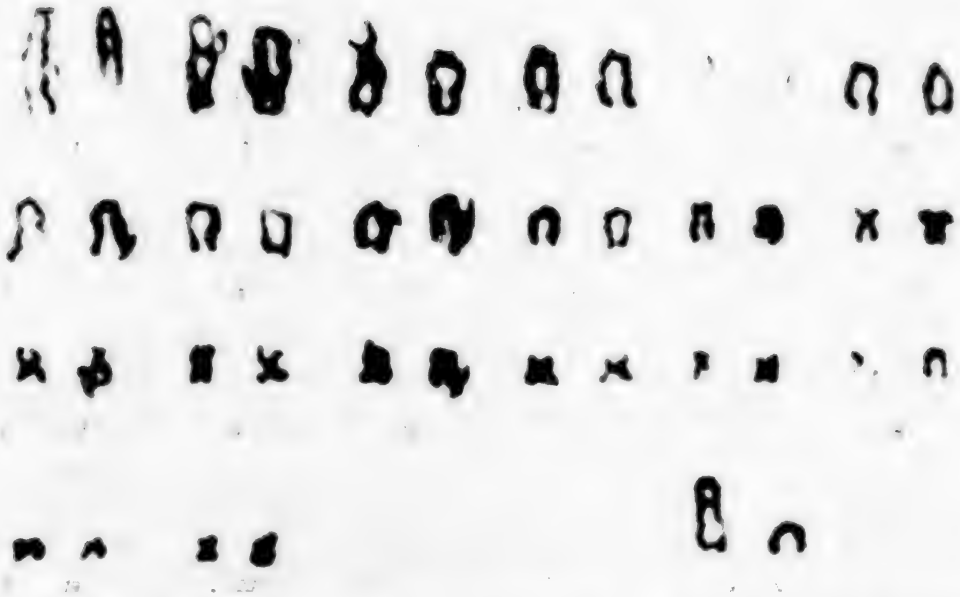

Figure 2. Karyotyping of rat chromosome. Translocation between first and third pairs (arrowheads)

It can be assumed that spaceflight factors can induce, to some extent or other, chromosome aberrations in bone marrow cells, the sensitivity of which to various exogenous factors is well-known. Such an effect was observed in chromosomes of bone marrow cells of rats flown aboard Cosmos-936 biosatellite. Damage to the genetic structures of peripheral blood lymphocytes was found [5, 7] in studies of over 18,000 metaphase plates before and after 14 manned flights. There was an increase in aberrations of the chromatid type in lymphocites. N. N. Bobkova and T. N. Krupina [1] failed to observe a reliable increase in frequency of aberrations before and after manned spaceflights in several spacecraft of the Soyuz type. However, in 3 out of 14 subjects, 1 dicentric chromosome was demonstrated, whereas after the flight such an aberration was found in only 1 case.

Thus, weightlessness had no appreciable influence on the mutagenic effect. It is difficult to determine, on the basis of available data, which of the extreme spaceflight factors plays the most substantial part.

However, when forecasting the risk of spaceflights, one must bear in mind that the aggregate of extreme factors could have a mutagenic effect.

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UDC: 616.72-009-02:612.766.2:629.78

DYNAMICS OF MORPHOLOGICAL CHANGES IN ARTICULATION NERVOUS SYSTEM UNDER HYPOKINETIC CONDITIONS AS A MODEL OF A SPACEFLIGHT FACTOR

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 18, No 4, Jul-Aug 84 (manuscript received 23 Feb 83) pp 43-49

[Article by V. I. Drobyshev, V. V. Antipov and V. V. Makarov]

[English abstract from source] Using statistical treatment, a neuromorphological examination of the joint capsule of rats exposed to hypokinesia for 7, 15, 20, 30, 40 and 60 days was carried out. The exposure to 7 days caused an increase in the number of reactively changed nerve fibers and receptors. The exposure to 15 days resulted in a significant increase of the number of nerve fibers with destructive changes that involved mostly large-caliber fibers. However, 20- and, especially, 30-day hypokinesia was followed by a significant reduction of destructive changes. After 40- and 60-day exposure they again became very distinct. This suggested a wave-like pattern of structural changes. At every stage of experimental hypokinesia all the compartments of the joint nervous apparatus showed adaptive-compensatory reactions.

[Text] Automation and complex mechanization of industrial processes, improvement of living conditions and consequent change in the life style of modern man cause significant decrease in energy expenditure and physical loads, which determine, to some extent, onset of a general biological problem of our century, hypokinesia. Problems of hypokinesia are acquiring particular significance in connection with space exploration and, consequently, man's exposure to weightlessness.

It is known that the skeletomuscular system is the first to react to weightlessness [2, 4], and one of the elements of this system are articulations with different receptor structures that perform a rather important role in mechanisms of sensory correction of voluntary movements [12, 23] and construction of the locomotor act [1, 17]. This is confirmed by representation of articular sensibility in the sensorimotor zone of the cerebral cortex [8, 21].

In the report of N. N. Il'yenko [10], who investigated articular receptors of the foreleg of 6 dogs, the movements of which were restricted for 3 months and

1 year, it was indicated that the types of receptors in the animals' articular capsule, their structure and dimensions are analogous to those found in the joint capsule of animals in the control group.

However, in spite of the substantial role of the articular nervous system in vital functions of man, the question of its state under the effect of hypokinesia, which causes a "shortage" of proprioceptive afferentation, is still unclear. This is one of the principal causes of the adverse effect of limited movement on man [16]. This work deals with investigation of these matters.

Methods

The nervous system, consisting of various structural components (capsule, articular labrum, cartilage, menisci, intraarticular ligaments), of 378 large joints of the extremities (shoulder, elbow, knee) from 126 rats, which were kept in special box-cages that restricted movement and did not allow them to move in space for 7, 15, 20, 30, 40 and 60 days (6 animals for each period) served as the object of our study. After conclusion of the experiment, the animals were switched to the usual upkeep in the vivarium and sacrificed using ether vapor on the 1st, 7th and 15th days of the recovery period. The experimental material was fixed in 12% neutral formalin followed by impregnation of the articular nervous system by the method of Kampos as modified by A. S. Shubin [15]; in some cases, the preparations were treated with 1% gold chloride. The condition of myelin sheaths of articular neural conductors was examined by the method of Sokolyanskiy [15].

Statistical processing according to R. B. Strelkov [19] was used to objectivize the observed morphological changes in neural conductors with small (1-3 μm) and large (3-7 μm or more) diameters at different stages of experimental hypokinesia and in the recovery period. The caliber of neural conductors was measured using an MOV-1-15 ocular micrometer. Determination was made of mean size (M), its error (m), amplitude of variation series (a) and coefficient (R) for 6 examined objects, which yielded reliability of $P<0.05$ and confidence interval ($L = a \cdot R$), as well as reliability ($100\% - L/M =$ %).

Results and Discussion

The studies of the nervous system of large joints of extremities of rats whose motor activity was limited revealed that, already after 7 days of hypokinesia there was an increase in number of neural conductors with reactive changes in the form of massive accumulation of neuroplasm, swelling of axis cylinders with signs of hypoargentophilia.

At all stages of experimental hypokinesia, the most marked reaction of the articular nervous system was demonstrable in preterminal segments of neural conductors. Numerous findings of neuromorphological studies [6, 13] are indicative of the high sensitivity of preterminal elements to diverse stimuli.

After 7-day hypokinesia, the terminals of articular receptors remained essentially unchanged, whereas after 15 days there were reactive changes, such as accumulations of neuroplasm and swelling and, in some cases, disintegration of

terminal ramifications. And, while 7-day hypokinesia caused some increase in number of neural conductors with reactive changes and their morphological signs corresponded to the level of the biological control after 1 week of recovery period, with increase in duration of restricted mobility to 15 days some of the neural conductors underwent destructive changes, which extended chiefly to afferent large-caliber conductors ($P<0.05$).

Experimental studies [22] have shown that the nervous system is quite sensitive to different factors, to which the body is exposed. It was established that, if the adaptive capacities of the articular nervous system are exceeded, it leads to destructive changes, as we observed after 15-day hypokinesia.

With 20-day hypokinesia, we observed an increase in share of reactively altered nerve elements. Concurrently, we demonstrated a decrease in number of neural conductors with destructive changes ($P<0.05$). This trend was the most distinct after 30-day hypokinesia (Figure 1).

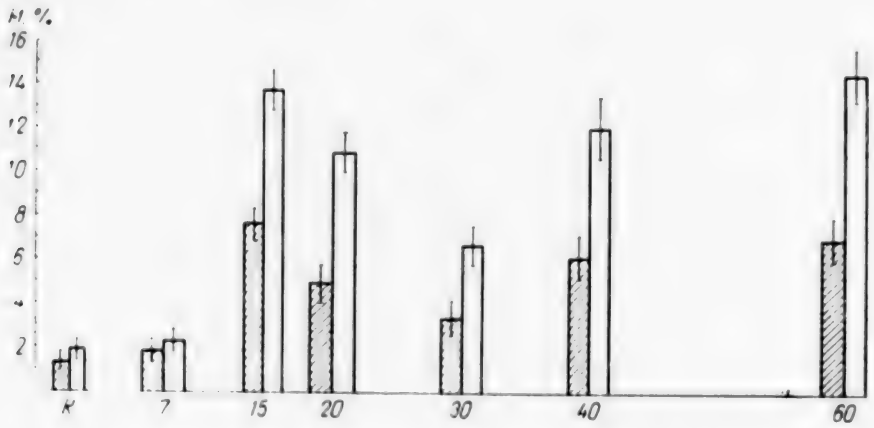

Figure 1. Dynamics of destructive changes in articular neural conductors with large (white bars) and small (striped) caliber during hypokinesia.

X-axis, time of examination; y-axis, number of neural conductors (%); K--control.

There are data in the literature [18] indicative of the fact that, after 7-day immobilization of a rabbit limb in a plaster cast, there are signs of irritation of articular nerve elements, and it is only after 15 days of this experimental factor that, along with reactive changes, destructive ones were also encountered, the frequency of which diminished after 1-month immobilization.

With further increase in duration of experimental hypokinesia to 40 and 60 days, we again observed an increase in number of destructively altered articular nerve fibers, mainly referable to those with a large caliber. There were more marked changes in the nervous system of the knee joint.

Thus, the morphological changes in the nervous system of large joints in the rat extremities, which were demonstrated throughout the experiment, fluctuated (were phasic) (see Figure 1).

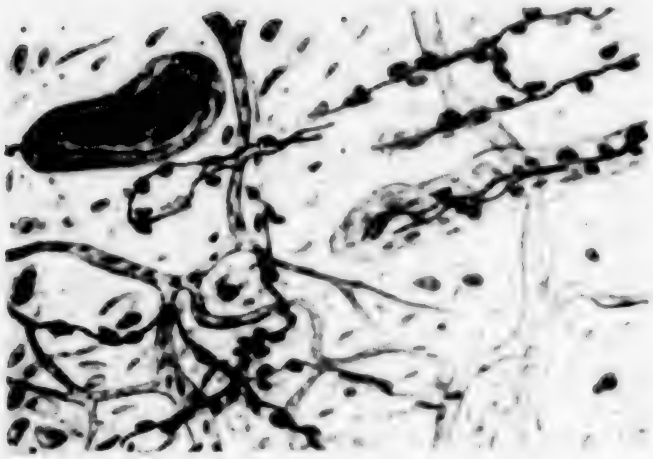

Figure 2.
Rat's knee capsule on 1st day after 15-day hypokinesia
Macroreceptor field. Impregnation according to Shubin
Ocular 12.5×, objective 40×

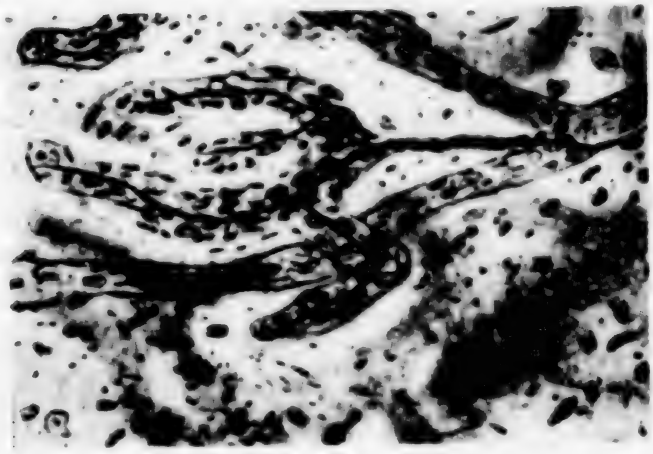

Figure 3.
Rat's shoulder joint capsule on 7th day after 30-day hypokinesia
"Budding" encapsulated nerve endings. Impregnation according to Shubin
Ocular 12.5×, objective 40×

Figure 4.
Rat's knee joint capsule on 7th day after 30-day hypokinesia
Collateral regeneration of neural conductors. Impregnation of neural conductors according to Shubin
Ocular 12.5×, objective 40×

Phasic changes were found in morphological composition of blood, bone marrow, spleen and metabolism of rats during long-term hypokinesia [11].

According to Ye. I. Il'ina-Kakuyeva [9], destructive changes developed in the motor plates of the rat soleus from the 7th to 30th day of restricted motor activity; after 30 days they were essentially of a repair nature.

Consequently, as indicated by our data and the literature, changes in nerve conductors of the locomotion system (muscles of extremities, joints) under hypokinetic conditions fluctuate (are phasic).

The process of destruction of articular elements ultimately changes to partial denervation of an organ or tissue which, in turn, causes development of recovery processes aimed at restoring the nervous system of joints that had lost its structural integrity. Such processes are referable to the category of compensatory adaptations [1, 14].

Compensatory and adaptive reactions, which we observed throughout the period of experimental hypokinesia, also fluctuated (were phasic). Already after 7 days of hypokinesia, there was proliferation of some terminal elements in the form of gradually disappearing end ramifications. We observed distinct swelling of neuroplasm with early signs of collateral regeneration. Some terminals of encapsulated nerve endings demonstrated a convoluted course and growth hillocks.

The spread of destructive changes after 15-day hypokinesia, in turn, caused compensatory reactions by the articular nervous system. We encountered collateral regeneration of nerve conductors with proliferation of the minutest nerve fibrils. There was proliferation of terminal branches of free nerve endings with formation of arboriform receptors. In this period we observed a decrease, as compared to 7-day hypokinesia, in encapsulated receptors characterized by tortuous terminals, presence of varicose thickening, accumulation of neuroplasm, growth hillock and additional branching.

Free and nonfree nonencapsulated and encapsulated receptors with well-visible compensatory and adaptive reactions in their terminals formed in the joint capsules microreceptor fields (Figure 2) that provided a specific level of proprioceptive information to cortical centers of the kinesthetic analyzer in the presence of diminished afferentation.

In addition to diminished level of destruction of nerve elements after 20-day hypokinesia, there was greater spread of compensatory and adaptive processes, which increased the most when the 30th day of restricted motor activity was reached. By this time, along with numerous proliferations of terminal branches of free and nonfree nonencapsulated nerve endings, we found many encapsulated receptors. The increase in the latter occurred as a result of "budding" and growth of sacculated terminals during hypokinesia and the recovery period (Figure 3). One of the distinctions of 30-day hypokinesia was arrangement of nerve endings in groups, with formation of microreceptor fields consisting of receptors differing in form. Evidently, such receptor fields provide, in the case of diminished afferentation, rather strong impulsation to the central nervous system for optimum activation of cortical fields of the kinesthetic

analyzer, whereas the impulses emitted by individual receptors to the centers are of subliminal value and do not elicit any responses [20].

There was an increase in number of unchanged nerve conductors after 30-day hypokinesia. At this time, there was wide distribution of collateral regeneration of nerve conductors with numerous proliferations of axis cylinders, which formed additional branching. We often encountered growth zones along the course of such conductors, in the form of neuroplasm bouton accumulation giving off the minutest fibrils in the form of gradually disappearing terminal ramifications.

We encountered regenerative nerve conductors parallel to destructively altered nerve fibers (Figure 4).

With increase in duration of hypokinesia from 40 to 60 days, there was gradual decrease in compensatory reactions of nerve elements. However, against the background of destructive changes, even in this period we observed many unchanged conductors and receptors, as well as nerve fibers characterized by a number of adaptive reactions in the form of accumulation of nerve substance along the course of axis cylinders, tortuosity of nerve conductors, additional branching, growth hillock which, in turn, provided for a larger area of contact with the innervated substrate.

Analysis of the condition of the articular nervous system in the recovery period revealed that the changes caused by short-term hypokinesia (7 days) underwent total reversal as early as the end of the 1st week. With increase in duration of restricted mobility to 15, 20 and particularly 30-60 days, recovery was slower. On the 7th day of the recovery period following 40- and 60-day hypokinesia, there was insignificant ($P>0.05$) decrease in number of altered conductors, and it is only on the 15th day that we observed a reliable decrease in number of altered conductors with retention of some afferent large-caliber nerve fibers with reactive changes. Consequently, the shorter the period of restricted activity, the sooner there is recovery of morphological structures. It can also be assumed that the continuing destruction of nerve elements in the recovery period following longer periods of experimental hypokinesia is attributable not only to preservation of products of degradation of previously destroyed nerve conductors, but apparently also to degradation of conductors that were formed during exposure to the extreme factor, i.e., the change to usual motor activity was, in turn, a "new" stimulus for newly formed nerve elements, which again caused some change in them. A change was also demonstrable in articular receptors, in particular, encapsulated ones. An increase in number of encapsulated receptors was observed from the 1st to 15th days of the recovery period. With increase in number of unchanged endings, there was increase in number of receptors with destructive changes, particularly on the 15th day of the recovery period. The "inertia" of productive processes in the recovery period is apparently due to the intensity of compensatory and adaptive processes aimed at restoring receptor elements that had lost their structural integrity. "Hyperproduction" of receptor elements during the hypokinetic period led to a greater volume of information in cortical centers, the need for which is eliminated during ordinary motor activity, so that there was an increase in receptors with destructive changes in the recovery period.

Thus, the animals' change to usual motor activity was also associated with changes in the articular nervous system, the severity of which depended on duration of experimental hypokinesia and of the recovery period. With increase in duration of the recovery period there was intensification of compensatory and adaptive reactions. The "hyperproduction" of receptor structures observed by the 7th day of the recovery period was followed, on the 15th day, by their partial destruction and a tendency of the receptor system to approximate the quantitative level of the biological control.

At the present time, hypokinesia is considered among the prime pathogenetic factors of weightlessness [3], which is also associated with restriction of motor activity and change in function of afferent systems [4], for which reason hypokinesia is used in simulating the effects of weightlessness [5].

Comparing the morphology of the articular nervous system under hypokinetic conditions and in weightlessness [7], it should be noted that the structural changes are in the same direction, in the form of nonspecific reactions--reactive and destructive changes in nerve elements. Well-marked compensatory and adaptive reactions in all elements of the articular nervous system were demonstrated in articular capsules. However, in the case of a long-term spaceflight, changes were noted in spatial organization of the articular nervous system in the form of disorganized, disoriented growth of nerve conductors, which could evidently be attributed to the absence of gravity in the flight experiment.

Thus, our findings are indicative of the diversity of morphological reactions of the articular nervous system to restriction of motor activity, the fluctuating (phasic) nature of these changes and rather well-marked compensatory and adaptive processes in all elements of the nervous system of joints. As compared to restriction of motor activity, the conditions of long-term spaceflights cause more marked structural changes with the spatial reorganization of articular nerve elements inherent in weightlessness.

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EFFECT OF 1,25-DIHYDROXYCHOLECALCIFEROL AND 24,25 DIHYDROXYCHOLECALCIFEROL ON GROWTH AND ALTERATION OF RAT BONES DURING HYPOKINESIA (HISTOMORPHOMETRIC STUDY)

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[Article by O. Ye. Kabitskaya, Z. F. Savik, A. S. Kaplanskiy, V. N. Shvets, I. N. Sergeyev, M. S. Belakovskiy and V. B. Spirichev]

[English abstract from source] The tubular bones of the fore- and hindlimbs of rats immobilized for 5 weeks were examined morphometrically and histologically. The rats were regularly given per os $1,25(OH)_2D_3$, $24,25(OH)_2D_3$ or their combination. The uptake of $24,25(OH)_2D_3$ at a dose of 1.25 μg or a combination of $1,25(OH)_2D_3$ and $24,25(OH)_2D_3$ at a dose of 0.03 + 0.25 μg led to the recovery of the linear and volume-weight rates of bone formation that changed during hypokinesia. However, these D_3 metabolites did not restore the width of the epiphyseal growth plate, whereas the size of the primary and secondary spongiosa returned to normal or exceeded it in response to $24,25(OH)_2D_3$ at a dose of 1.25 μg and $1,25(OH)_2D_3$ at a dose of 0.15 μg, respectively (only these two doses were used); in other words, the D_3 metabolites prevented osteoporosis which is typical of hypokinesia. It is assumed that hypokinesia may produce either disorders in D_3 metabolism or changes in the sensitivity of bone cells to active D_3 metabolites and other hormones that are directly or indirectly involved in osteogenesis regulation.

[Text] Formation, mineralization and resorption of bone tissue are regulated to a significant extent by vitamin D [10]. The original vitamin D does not have biological activity in itself, but it functions only after conversion to active metabolites--1,25-dihydroxycholecalciferol D_3--$1,25(OH)_2D_3$--and 24,25-dihydroxycholecalciferol D_3--$24,25(OH)_2D_3$ [7]. The main action of $1,25(OH)_2D_3$ in physiological concentrations is aimed at the mucosa of the small intestine, and the increase elicited by this metabolite in concentration of calcium in the interstitial space could provide for normal mineralization of bone and stimulate its formation, particularly in regions of bone resorption [13]. When the physiological level is exceeded, the effect of $1,25(OH)_2D_3$ on bone tissue is determined primarily by its resorptive activity [7, 10, 13].

In contrast, the metabolite $24,25(OH)_2D_3$ provides for utilization of calcium during mineralization and, perhaps, stimulates formation of bone tissue [1, 2, 8, 11, 13]. This metabolite retains the capacity to intensify absorption of calcium in the small intestine and has no marked resorptive effect on bone [7, 10].

We previously demonstrated that metabolism of vitamin D_3 changes in rats under hypokinetic conditions [4]. Preventive use of $24,25(OH)_2D_3$ under such conditions had a beneficial effect on chemistry of bone tissue [5], which could be related to the stimulating effect of this metabolite of D_3 on osteogenesis.

Our objective here was to investigate the possible prophylactic effect of functionally active metabolites of vitamin D_3 on growth and alteration of bones under hypokinetic conditions.

Methods

In the experiments we used male Wistar rats with initial weight of 240-280 g. The animals received a semisynthetic diet containing vitamin D, 0.6% calcium and 0.6% phosphorus [6]. Hypokinesia was produced by keeping the rats in adjustable cages. We gave $1.25(OH)_2D_3$ and $24,25(OH)_2D_3$ separately or in combination daily, by mouth, in 0.1 mℓ propylene glycol. The doses of metabolites of vitamin D_3 used in the experiment are listed in Table 1. The smaller of these doses for each metabolite is physiological for rats [1]. We tested 8-10 animals in each group. The rats were kept in hypokinetic cages for 5 weeks.

Table 1.
Evaluation of efficacy of vitamin D_3 metabolites on bone growth parameters

Vitamin metabolites	Dosage, μg	Parameter			
		growth in length	growth in width	volume increase	mass increase
$1,25(OH)_2D_3$	0.03			·	·
$1,25(OH)_2D_3$	0.15			+	
$24,25(OH)_2D_3$	0.25				
$24,25(OH)_2D_3$	1.25				
$1,25(OH)_2D_3$	0.03				
$24,25(OH)_2D_3$	0.25				
$1,25(OH)_2D_3$	0.03		±	·	
$24,25(OH)_2D_3$	1.25				

Key:
++) maximum beneficial effect
+) beneficial effect
±) possible beneficial effect
-) no effect

To assess rat bone growth under hypokinetic conditions we used morphometric parameters. Calipers were used to measure bone length. This parameter is an integral indicator of lengthwise bone growth. Since the bones of constantly growing animals grow not only in length but in width, we also used the latter parameter, which is indicative of periosteal and endosteal osteogenesis and bone resorption. Formation and resorption of bone was assessed by the change in area of the cortical plate and bone marrow canal. For this purpose, the bones of anterior and posterior extremities were fixed in 10% formalin, decalcified in 5% nitric acid, and sections about 20 μm thick were prepared on a freezing microtome. The tibia was cut at a right angle to its axis in the region of articulation with the fibula, and three successive sections were prepared from the proximal segment. The humerus was cut distal to the tuberositas deltoideus. A photo enlarger was used to project sections on paper, they were outlined with pencil and each

section was submitted to planimetry three times. In addition, we measured the volume of diaphyseal and distal epiphyseal segments of the femur, their dry weight after defatting and density [6].

The tibia was used to evaluate endochondral bone growth. After fixing the bone in 10% formalin, it was decalcified in 10% EDTA (pH 7.0) and imbedded in Histoplast. Serial sections, 7 μm in thickness, were prepared parallel to the frontal plane; they were stained with hematoxylin and eosin, picrofuchsin, by the Brachet method, toluidine blue and alcian blue (pH 2.8). The morphogravimetric method was used to measure the thickness of the chondral growth plate of the proximal epiphysis of the tibia. In addition, using an ocular micrometer, we measured the thickness of the plate, zone of chondral cell columns and zone of swelling and decalcification of cartilage. The relative volume of osseous trabeculae of the primary and secondary spongiosa in the proximal end of the tibia was determined on negative images of the bone using a 5-mm test grid. Osteoblasts were counted in the region of the primary spongiosa in 15 fields of vision at magnification of 900×.

Results and Discussion

The rate of lengthwise bone growth decreased with statistical reliability ($P<0.001$) during hypokinesia, as compared to the control and remained at about the same level with intake of $1,25(OH)_2D_3$ (Figure 1a). With use of $24,25(OH)_2D_3$, the rate of lengthwise bone growth was restored to almost the control level. A dosage of 1.25 μg was found to be the most effective. A combination of the two vitamin D_3 metabolites in physiological doses also had a beneficial effect.

There was a similar direction of changes in bone growth in width (Figure 1b-d). Thus, while the cross section decreased by 6-8% under hypokinetic condition, as compared to the control (statistically unreliable differences) and this decrease persisted with intake of $1,25(OH)_2D_3$, intake of $24,25(OH)_2D_3$ in a dosage of 0.25 μg or of a combination of this metabolite in a dosage of 0.25 μg with $1,25(OH)_2D_3$ in a dosage of 0.03 μg led to total restoration of cross section width (see Figure 1b). The area of the cortical plate of the diaphysis of the humerus and tibia (see Figure 1d) decreased under hypokinetic conditions with statistical reliability ($P<0.001$), and remained about the same with intake of $1,25(OH)_2D_3$, whereas when it was combined with $24,25(OH)_2D_3$ the adverse effect of hypokinesia was eliminated. The deciding role was played by $24,25(OH)_2D_3$, since intake of it alone, in a dosage of 1.25 μg, led to restoration of this bone parameter. Thinning of the cortical plate occurred due to enlargement of area of the bone marrow canal (see Figure 1c). Such changes were particularly inherent in the tibia and less so in the humerus. With all variants of giving metabolites of vitamin D_3, the area of the medullary canal of the tibia was 5-20% larger, while that of the humerus either increased by 3-11% or decreased by 13-14%, as compared to the control.

To sum up the results of measurement of linear parameters of bone, it can be concluded that $24,25(OH)_2D_3$ in a dosage of 1.25 μg, or a combination of $1,25(OH)_2D_3$ with $24,25(OH)_2D_3$ in doses of 0.03 and 0.25 μg, respectively, have a preventive effect on bone growth in length and width. Other combinations of vitamin D_3 metabolites either eliminated the effect of hypokinesia partially or had no beneficial effect whatsoever.

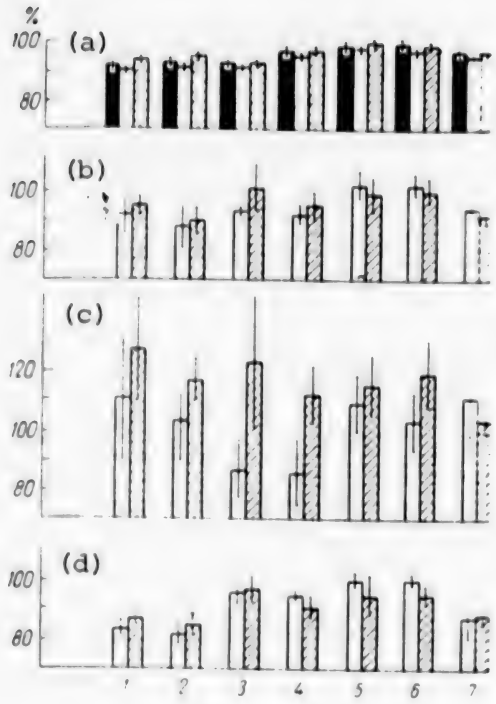

Figure 1.
Effect of $1,25(OH)_2D_3$ and $24,25(OH)_2D_3$ on linear rate of osteogenesis in hypokinetic rats (% of control)
a) length
b) cross-section area
c) area of medullary canal
d) area of cortical plate
Here and in Figure 2:
1) hypokinesia
2) hypokinesia + $1,25(OH)_2D_3$ (0.03 μg)
3) hypokinesia + $1,25(OH)_2D_3$ (0.15 μg)
4) hypokinesia + $24,25(OH)_2D_3$ (0.25 μg)
5) hypokinesia + $24,25(OH)_2D_3$ (1.25 μg)
6) hypokinesia + $1,25(OH)_2D_3$ and $24,25(OH)_2D_3$ (0.03 and 0.25 μg)
7) hypokinesia + $1,25(OH)_2D_3$ and $24,25(OH)_2D_3$ (0.03 and 1.25 μg)
Black bars refer to femur, white to humerus and striped to tibia.

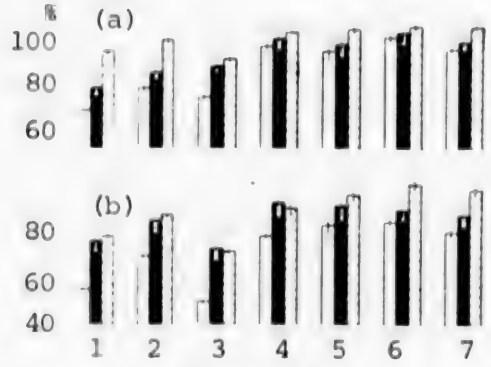

Figure 2.
Effect of $1,25(OH)_2D_3$ and $24,25(OH)_2D_3$ on weight, volume and density of diaphyses (a) and distal epiphyses (b) of femur in hypokinetic rats (% of control)
White bars--weight, black--volume, striped--density

Depression of bone growth in hypokinetic rats was demonstrable when determination was made of weight ($P<0.001$) and volume ($P<0.001$) of diaphyseal and epiphyseal parts of the femur (Figure 2). We observed a decrease in their density ($P<0.01$) due to more significant weight drop determined by mineralization of bone tissue, as compared to volume. The best recovery of tested parameters was observed with use of $24,25(OH)_2D_3$ in a dose of 1.25 μg and particularly the combination of $1,25(OH)_2D_3$ and $24,25(OH)_2D_3$ (0.03 + 0.25 μg). With use of $1,25(OH)_2D_3$ we observed a tendency toward restoration of volume and mass of diaphyses; however, when the dosage of this metabolite was increased, diaphyseal density decreased, as compared to hypokinetic animals, probably due to resorption of de novo formed bone tissue. A beneficial effect of $1,25(OH)_2D_3$ on the epiphyses was observed only with use of a physiological dosage, whereas a higher dose of this metabolite did not stimulate growth of epiphyses and apparently intensified somewhat their resorption.

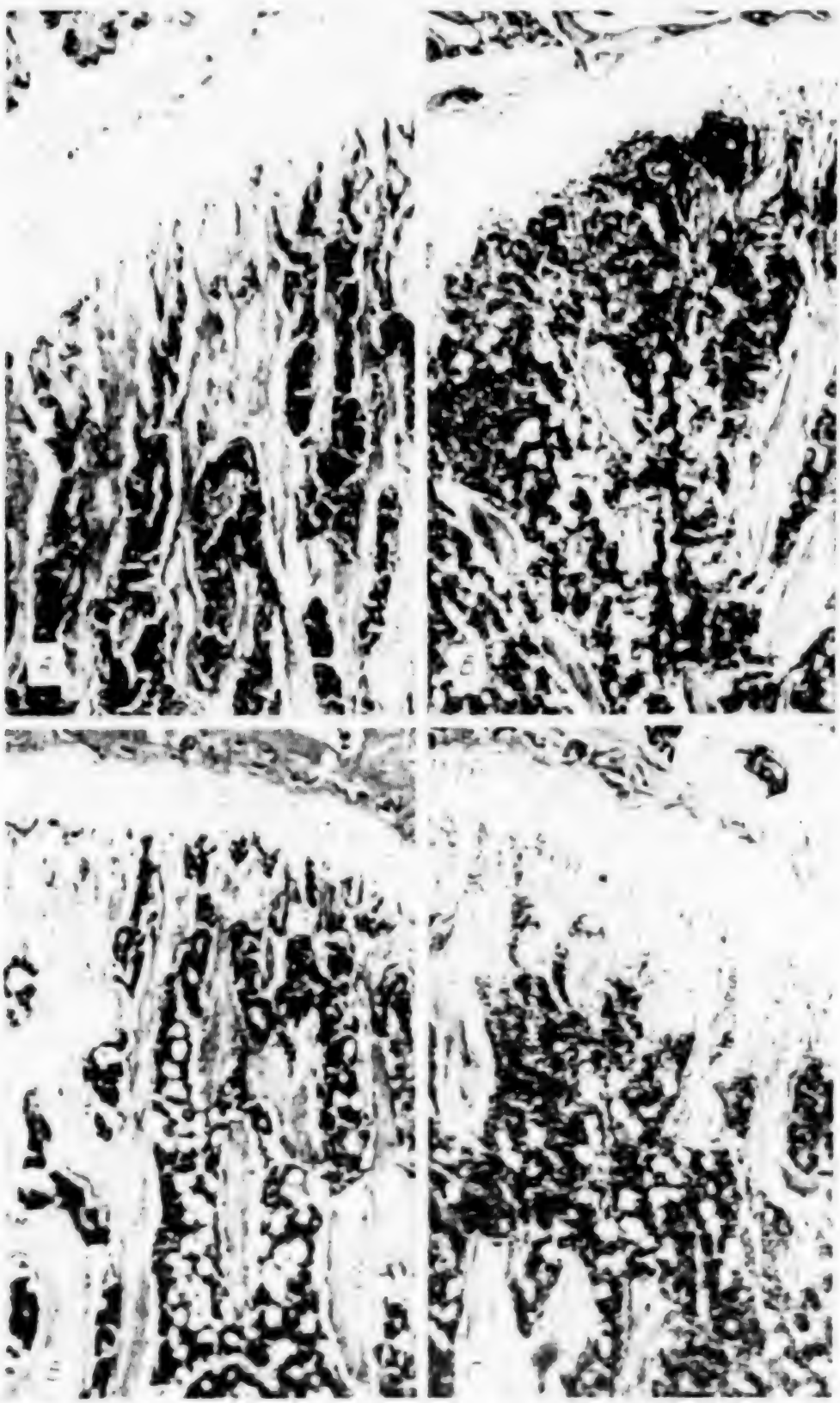

Figure 3. Tibial epiphyseal growth plate and metaphyseal spongiosa.
А) control; Б) hypokinesia (reduced growth plate thickness, primary and secondary spongiosa markedly thinned, their volume reduced) В) hypokinesia + 24,25$(OH)_2D_3$, 1.25 μg (reduced growth plate thickness, volume of primary and secondary spongiosa close to control); Г) hypokinesia + 1,25$(OH)_2D_3$, 0.15 μg (reduced thickness of growth plate and volume of primary and secondary spongiosa). Hematoxylin and eosin stain, 3.5× lens, 7× eyepiece

Arbitrary evaluation of efficacy of the vitamin D_3 metabolites used on integral parameters of bone growth is listed in Table 1.

Table 2.
Thickness of cartilage (epiphyseal) growth plate of rat tibia according to results of morphogravimetric measurement

Animal group	Thickness of growth plate	
	arbitr. units	%
Control	70,7±3,62	100
Hypokinesia	45,3±4,41*	64
Hypokinesia + $1,25(OH)_2D_3$(0,15 μg)	42,3±2,10*	60
Hypokinesia + $24,25(OH)_2D_3$(1,25 μg)	43,9±1,62*	62

Note: Here and in Tables 3-4, asterisk marks statistically reliable differences between experiment and control.

During hypokinesia, there was not only inhibition of bone growth, but osteoporosis of spongy bone spongiosa [3, 9]. In this regard, the question arises as to whether there is change in structure of the epiphyseal growth plate under the prophylactic effect of D_3 metabolites. To answer this question, we undertook a morphometric and histological study of the spongiosa and epiphyseal growth plate in rats given a vitamin D enriched diet and additional amounts of active metabolites of this vitamin. According to the data listed in Tables 2 and 3, the thickness of the chondral growth plate was considerably smaller in hypokinetic rats than control animals of the same age. As shown by morphometric studies (see Table 3), the decrease in thickness of the growth plate occurred due to narrowing of both the zone of chondral cell columns and zone of swelling and calcification of cartilage, as a result of which there was no change in correlation between the different zones in the chondral growth plate. The intensity of staining of the basic substance of the growth plate cartilage with alcian blue decreased somewhat in hypokinetic rats, which was indicative of decrease in amount of acid mucopolysaccharides in the cartilage. At the same time, there was also a decrease in ribonucleoprotein content of chondrocyte cytoplasm, which was indicative of inhibition of synthetic processes in the cells. The weight of the primary spongiosa in the immediate vicinity of the chondral growth plate, as well as secondary spongiosa, in rats submitted to hypokinesia decreased significantly, and this was due to both shortening of osseous trabeculae and their thinning (Table 4, Figure 3a and 3b). There was a decrease in number of osteoblasts in the region of the primary spongiosa in hypokinetic rats (see Table 3). The osteoblasts no longer presented an orderly arrangement on the surface of osseous trabeculae and collected into small groups in the spaces separating the trabeculae. There was a decrease in pyroninophilia of osteoblast cytoplasm and in its volume. We failed to demonstrate substantial disturbances referable to arrangement or decrease in number of osteoblasts lining the endosteal bone surface. Hypokinetic rats also presented adiposity of red bone marrow and increase in number of mast cells in it.

Giving hypokinetic rats $1,25(OH)_2D_3$ or $24,25(OH)_2D_3$ had no noticeable effect on thickness of the chondral growth plate and its different zones, or on number of osteoblasts in the region of the primary spongiosa (see Tables 2 and 3), intensity of alcianophilia of basic chondral substance and pyroninophilia of cytoplasm of chondrocytes and osteoblasts. At the same time, as seen from the

data listed in Table 4, the relative volume of primary and secondary spongiosa in rats given $24,25(OH)_2D_3$ was the same as in control animals, whereas in rats given $1,25(OH)_2D_3$, although it was smaller than in the control it exceeded the value for rats submitted to hypokinesia (see Figure 3c, 3d). Normalization of relative volume of primary and secondary spongiosa of rats given $24,25(OH)_2D_3$ occurred mainly as a result of increased thickness and dimensions of osseous trabeculae, rather than in their number, and this was probably related to stimulation by this D_3 metabolite primarily of bone tissue mineralization. Such an ossifying effect of $24,25(OH)_2D_3$ prevented development of spongy bone osteoporosis; however, its structure became coarser. The structural change in spongy bone, which was associated with a decrease in number of osseous trabeculae with concurrent coarsening of remaining ones, could affect the strength characteristics of bone. In a larger dose, $1,25(OH)_2D_3$ also stimulated osteogenesis; however, there was apparently prevalence of resorption of de novo formed spongiosa. As a result, osteoporosis of spongy bone was somewhat less marked in these animlas than in hypokinetic rats.

Table 3. Parameters of tibial epiphyseal growth plate

Animal group	Growth plate thickness, mm	Thickness of chondr. cell column zones, mm	Thickness of zone of chondral swelling & calcification, mm	Number of osteoblasts
Control	0,21 ± 0,009	0,10 ± 0,006	0,09 ± 0,007	36 ± 1,2
Hypokinesia	0,15 ± 0,01*	0,7 ± 0,002*	0,07 ± 0,004*	21 ± 1,4*
Hypokinesia + $1,25(OH)_2D_3$ (0.15 µg)	0,16 ± 0,006*	0,08 ± 0,002*	0,07 ± 0,002*	21 ± 1,5*
Hypokinesia + $24,25(OH)_2D_3$ (1.25 µg)	0,16 ± 0,007*	0,07 ± 0,003*	0,07 ± 0,002*	25 ± 2,4*

Table 4.
Relative volume of primary and secondary spongiosa in proximal segment of tibia

Animal group	Spongiosa volume, %
Control	9,9 ± 0,5
Hypokinesia	7,1 ± 0,4*
Hypokinesia + $1,25(OH)_2D_3$ (0.15 µg)	8,8 ± 0,9
Hypokinesia + $24,25(OH)_2D_3$ (1.25 µg)	10,0 ± 0,6

Thus, $1,25(OH)_2D_3$ and $24,25(OH)_2D_3$ did not help restore all of the tested bone parameters that were altered under hypokinetic conditions. The only exceptions were linear and volume-weight parameters of bone growth, whereas the histological parameters of spongy bone differed appreciably from those of intact animals. Such a dissimilar effect of vitamin D_3 metabolites has not yet been explained. We cannot rule out the possibility that compact and spongy bone function as separate components, which also have different mechanisms for maintaining homeostasis [12, 14]. Nevertheless, the general effect of $24,25(OH)_2D_3$ on bone is apparently related to intensification of osteogenesis and mineralization without appreciable influence on bone tissue resorption, whereas $1,25(OH)_2D_3$ probably accelerates

its alteration. No doubt, the effect of D_3 metabolites on bone tissue is mediated, to some extent, by their effect on calcium metabolism.

The results of using functionally active vitamin D_3 metabolites lead us to the preliminary conclusion that depression of bone growth in hypokinetic rats may be related to altered production of $1,25(OH)_2D_3$ and $24,25(OH)_2D_3$ in the body and/or sensitivity of bone tissue not only to these metabolites, but to a number of hormones directly or indirectly involved in regulation of histogenesis of bone. It is quite obvious that the question of possible prophylactic effect of $1,25(OH)_2D_3$ and $24,25(OH)_2D_3$ on bone metabolism, when mineral metabolism is impaired under hypokinetic conditions, requires further investigation.

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DYNAMICS OF SOME PARAMETERS OF CARBOHYDRATE AND LIPID METABOLISM IN RECOVERY PERIOD FOLLOWING LONG-TERM HYPOKINESIA

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 18, No 4, Jul-Aug 84 (manuscript received 18 Mar 83) pp 55-58

[Article by T. M. Lobova, P. P. Potapov and A. V. Chernyy]

[English abstract from source] Eighty-seven white rats were exposed to prolonged hypokinesia. On 90th hypokinesia day the content of cholesterol, free fatty acids and acetone bodies increased and the content of sugar and triglycerides decreased in blood, the content of glycogen decreased and the content of cholesterol increased in liver and skeletal muscles. On the 15th day after exposure most parameters returned to normal. However, glucose-6-phosphate dehydrogenase in liver and adipose tissue increased and remained elevated till recovery day 60. On the 30th recovery day the changes were similar to those during hypokinesia. On the 90th recovery day the content of triglycerides, cholesterol and acetone bodies in blood grew and the content of triglycerides and glycogen in muscles increased.

[Text] Restriction of motor activity can lead to substantial disturbances in carbohydrate and lipid metabolism [4, 7, 9-11]. The question of possibility and time of normalization in the recovery period has been insufficiently explored. The published data pertain only to blood lipid levels [1, 4, 7], and in a number of instances more appreciable changes were demonstrated than under hypokinetic conditions. Our objective here was to study the parameters of ... [omission in source] and lipid metabolism in blood and tissues during the recovery period following 90-day hypokinesia.

Methods

This study was conducted on 87 white male rats with initial weight of 180-220 g. The animals were placed in small individual cages made of plexiglas. Control animals were kept under the usual vivarium conditions. The rats were decapitated on the 90th day of hypokinesia and on the 15th, 30th, 60th and 90th days of the recovery period. We assayed glycogen [14], total lipids (TL), cholesterol (CS) and triglycerides (TG) [2] in hepatic tissues and skeletal

muscles, and activity of glucose-6-phosphat (EC 1.1.1.49) [6] in the liver and fatty tissue. We measured blood serum sugar, total CS, CS of high-density (α-CS) and low-density (β-CS) lipoproteins [12], acetone bodies (AB) [11] and free fatty acids (FFA) [13].

Results and Discussion

On the 90th day of hypokinesia experimental rats showed significant elevation of CS, FFA and AB levels in blood, moderate hypoglycemia and hypotriglyceridemia (Table 1). In the liver, TL and CS levels increased by 30 and 31%, respectively ($P<0.01$). Glycogen content dropped and constituted only 18% ($P<0.01$) of the control level (Table 2). Similar, but less marked, changes were demonstrated in skeletal muscle tissue. Evidently, acids are the principal source of energy under hypokinetic conditions. However, in view of the limited capacity of tissues to utilize active acetate, it is intensively involved in CS synthesis [8].

On the 15th day of the recovery period there was a distinct tendency toward normalization of most of the parameters studied. There was significant decrease (but not to normal values) in FFA and AB levels in blood, blood sugar and TG, glucogen in the liver and skeletal muscles, TL and CS in the liver were close to base levels. TG content of hepatic tissue decreased somewhat. At this time, there was drastic increase in TL content of skeletal muscles, as compared to both the control and 90th day of hypokinesia. It is most probable that this increase was attributable to phospholipids, since there was insignificant change in TG and CS levels.

The trend toward recovery observed on the 15th day of the recovery period for most parameters was transient. One month after the animals returned to ordinary living conditions, changes reappeared that were virtually the same as those observed on the 90th day of hypokinesia. They were most probably attributable to the inconsistency between increasing motor activity and anabolic capabilities of energetically important substrates. Evidently, the regulatory mechanisms mobilized by the body during the period of readaptation, which are aimed at maintaining homeostasis, were inadequate when muscular activity increased. It can be maintained that the return to base values of some metabolites in tissues at the early stages of the recovery period is not yet proof of normalization of metabolism as a whole. Disturbances (mainly in enzymatic activity) persisted for a long time. The impression was gained that the new "metabolic stereotype," which was formed during hypokinesia and the important distinctions of which are intensification of liposynthesis and utilization of fatty acids as the chief source of energy, was quite persistent. Intensification of fatty acid synthesis in rats during the recovery period was corroborated by the substantial activation of dehydrogenase reactions of the pentose-phosphate route of glucose oxidation in fatty tissue and the liver on the 30th and 60th days of the recovery period (Table 3) and more intensive deposition of lipids in the fat reservoirs and skeletal muscles.

Progressive fat deposition continued on the 90th day of the recovery period. At this time, experimental animals weighed more (by 11%) than control rats, although weight was 40% lower on the 90th day of hypokinesia. Interestingly, an increase in body weight in the period of recovery after prolonged hypokinesia has also been observed in humans, in a number of instances [3].

Table 1. Rat serum lipids, blood AB and sugar in recovery period after 90-day hypokinesia (M±m)

Parameter	Control	Hypokinesia (90 days)	Day of recovery period		
			15	30	45
TG, mg%	52.3 ± 2.6 (24)	43.8 ± 1.0 (8)*	53.6 ± 3.6 (6)	38.8 ± 2.6 (8)*	62.0 ± 3.4 (7)*
CS, mg%	69.5 ± 1.6 (24)	98.5 ± 3.2 (8)*	93.8 ± 2.3 (8)*	90.4 ± 1.3 (8)*	81.5 ± 5.0 (9)*
FFA, μeq/ℓ	210 ± 20 (15)	480 ± 40 (5)*	320 ± 33 (11)*	410 ± 57 (16)*	
AB, mg%	0.150 ± 0.026 (10)	0.970 ± 0.140 (5)*	0.270 ± 0.030 (8)*	0.756 ± 0.165 (16)*	0.301 ± 0.011 (7)*
Sugar, mg%	98.0 ± 1.8 (10)	75.0 ± 1.6 (10)*	103.0 ± 3.7 (8)	90.0 ± 2.3 (8)*	

Note: Here and in Tables 2-3, number of animals is given in parentheses. Asterisk indicates statistically reliable changes, in comparison to control ($P<0.05$)

Table 2. Rat tissue lipid and glycogen content in recovery period after 90-day hypokinesia (M±m)

Tissue	Parameter	Control	Hypokinesia (90 days)	Day of recovery period		
				15	30	45
Skeletal muscles	TL, g%	2.13 ± 0.12 (10)	2.08 ± 0.07 (5)	3.54 ± 0.40 (8)*	1.62 ± 0.18 (16)*	2.24 ± 0.14 (9)
	TG, mg%	388 ± 8 (24)	380 ± 9 (8)	364 ± 15 (8)	600 ± 12 (8)*	458 ± 7 (9)*
	CS, mg%	88.8 ± 1.1 (24)	104.7 ± 1.5 (8)*	100.0 ± 3.8 (8)*	92.5 ± 1.6 (8)	90.3 ± 2.8 (9)
	Glycogen, mg%	263 ± 65 (28)	443 ± 29 (13)*	560 ± 54 (12)	447 ± 30 (16)*	1393 ± 104 (9)*
Liver	TL, g%	4.07 ± 0.24 (10)	5.28 ± 0.20 (5)*	3.99 ± 0.08 (7)	4.77 ± 0.20 (8)*	3.66 ± 0.09 (9)
	TG, mg%	473 ± 7 (24)	450 ± 12 (8)	380 ± 8 (8)*	497 ± 14 (8)	480 ± 7 (9)
	CS, mg%	233.1 ± 9.9 (10)	307.0 ± 5.0 (5)*	223.2 ± 13.2 (7)	298.8 ± 7.9 (16)*	182.0 ± 9.0 (9)*
	Glycogen	4800 ± 325 (30)	571 ± 49 (13)*	5412 ± 692 (12)	2500 ± 298 (16)*	3033 ± 278 (9)

Table 3. Rat tissue glucose-6-phosphate dehydrogenase activity in recovery period after 90-day hypokinesia (in μM reduced NADP/g protein/min)

Tissue	Control	Hypokinesia (90 days)	Day of recovery period			
			15	30	45	60
Liver	5.03 ± 0.14 (21)	5.66 ± 0.33 (7)	5.55 ± 0.19 (8)*	5.85 ± 0.28 (8)*	5.96 ± 0.37 (7)*	5.10 ± 0.28 (9)
Adipose	28.9 ± 2.1 (19)	25.5 ± 3.4 (6)	45.1 ± 2.8 (8)*	67.0 ± 11.0 (8)*	54.9 ± 4.3 (7)*	42.1 ± 3.3 (9)*

On the 90th day of unrestricted motor activity, there was recovery to base values of glycogen, TL and TG levels in the liver, TL and CS in skeletal muscles, glucose-6-phosphate dehydrogenase activity in the liver and fatty tissue. There was distinct decline (but not to normal values) of blood AB and total CS levels. There was reliable increase in β-CS in blood (by 40.4%; $P<0.05$) and a tendency toward decrease of α-CS. The coefficient of atherogenicity rose by 46% ($P<0.05$). A distinctive super-recovery effect was found for several parameters, including hepatic CS, blood serum and skeletal muscle TG, fatty tissue lipids. Thus, excessive production of TG in the body, increase in β-CS and TL in blood must be viewed as adverse consequences of hypokinetic disorders.

At the present time, data have been accumulated concerning the existence of a distinct correlation between hyperlipidemia and incidence of cardiovascular disease [5]. For this reason, it is desirable to provide for a set of measures to be used in the recovery period following long-term hypokinesia in order to eliminate disturbances in lipid metabolism that form the metabolic background for onset and progression of diseases of the heart and vessels.

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BILIGENIC FUNCTION OF WHITE RAT LIVER

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 18, No 4, Jul-Aug 84 (manuscript received 15 Feb 83) pp 58-62

[Article by Ye. N. Panasyuk and L. N. Skakun]

[English abstract from source] White male rats were exposed to hypokinesia of 4 to 30 days in duration. The exposure led to a moderate hypersecretion of gall which was more distinct in autumn and winter. The synthesis and secretion of gallic acid increased. The excretion of cholesterol also grew. The ratio of tauro- and glycoacids shifted in favor of the latter. The cholate cholesterol coefficient increased. These changes in gall formation during hypokinesia are closely related to disorders in lipid and carbohydrate metabolism, as well as in adrenal function.

[Text] Metabolism, the structure and function of several organs and systems, including the liver, undergo changes when motor activity is restricted. In particular, the liver decreases in weight, there is impairment of histochemical and submicroscopic structure [1, 6, 13], ploidy of hepatocyte nuclei and their mitotic activity decrease, there is increase in number of binuclear cells [9]. There is drastic decrease in glycogen content of hepatocyte cytoplasm, RNA and DNA levels drop, activity of some serum enzymes (alanine aminotransferase, asparate aminotransferase, alkaline phosphatase) decreases, there is change in levels of bile acid, cholesterol and phospholipids in bile [11, 14, 16, 20, 21]. In a previous study, it was established that the effect on the liver of cholagogues changes under the effect of hypokinesia [17].

Our objective here was to test the effect of hypokinesia on biligenesis during the fall-winter and spring-summer periods. We devoted special attention to synthesis of bile acids and their conjugation with amino acids--taurine and glycine.

Methods

Experiments were conducted on 92 male, mongrel white rats weighing 140-170 g. Biligenic function of the liver was evaluated by the method of N. P. Skakun and A. N. Oleynik [18] on the 4th, 8th, 15th and 30th days of hypokinesia in the fall-winter and spring-summer. The experiments began with anesthetization

of the animals by means of intraperitoneal injection of 1% freshly prepared barbamyl solution, at the rate of 0.6-0.8 mℓ/100 g body weight. After immobilizing the rats, the abdominal cavity was opened in midline, the common bile duct was located and two fine ligatures pulled under it. One was tied in the distal part of the duct, after which an incision was made in it. A fine flexible polyethylene tube, 12-14 cm in length, was introduced to a depth of 5-6 mm in this incision and secured with the second ligature. A beaker was placed under the open end of this tube to collect bile. Then, 1-2 sutures were applied to the surgical wound. All of the experiments were performed at ambient temperature of 25°C in the laboratory. Bile was collected in 1-h batches for 4 h.

The following served as indicators of intensity of biligenesis: rate of bile secretion during the 4-h experiment (in mg/100 animal weight/min) and total amount of bile recovered during the experiment, i.e., in 4 h (in g/kg). We evaluated cholate production and cholate excretion according to the following parameters: total concentration of bile acids in bile (g/ℓ), concentration of bile acids conjugated with taurine and glycine (g/ℓ) and total bile acids, their taurine and glycine conjugates per experimental hour (g/kg). In all 1-h batches of bile, we determined the concentration (μmol/ℓ) and total amount (μmol/kg) of bilirubin and cholesterol. Bile acids in the bile were separated by ascending chromatography, followed by measurement of concentration and total quantity of bile acids conjugated with taurine (taurocholic acid) and glycine (glycocholic) [4, 5]. We assayed concentrations of cholesterol in bile by the method of S. M. Drogovoz [14] and bilirubin according to N. P. Skakun [19]. The numerical data were analyzed using Student's t criterion.

A model of hypokinesia was produced by placing the animals in special box-cages, in which their mobility was drastically restricted.

Results and Discussion

As can be seen from the data listed in Table 1, restriction of the animals' motor activity led to increase in rate of bile secretion. In the experiments conducted in the fall-winter (first series), secretion rate increased already on the 4th day on the average from 4.3±0.3-3.9±0.3 to 5.4±0.4-4.2±0.3 mg/g weight/min. As a result, total bile increased in the 4 h of the experiment from 9.90±0.46 to 12.00±0.63 g/kg, i.e., by 21%. On the 8th day, this parameter reached 12.72±0.40 g/kg and on the 15th day, 14.22±0.49 g/kg, i.e., it increased by 29 and 44%, respectively. By the 30th day, the intensity of biligenesis decreased to about the base level. In the second series of experiments, which were conducted in the spring-summer, the intensity of bile production increased by 14, 7 and 15% on the 8th, 15th and 30th days of hypokinesia, respectively.

Hypersecretion of bile under hypokinetic conditions occurred against a background of alteration of its chemistry, particularly levels of bile acids and cholesterol in bile (Table 2). There was an increase in concentration of bile acids at all stages of hypokinesia, particularly on the 8th day. By this time, total chelate concentration increased on the average from 10.53±0.95 to 21.34±2.73 g/ℓ, or by more than 2 times, in the first series and from 10.58±0.97

to 16.50±1.40 g/ℓ, or by more than 1.5 times, in the second series. Chromatographic analysis of bile revealed that the increase in overall concentration of bile acids is attributable to taurocholic and glycocholic acids (particularly the latter). As a result, the correlation of bile acids shifted in the direction of glyco acids at all tested times. Analogous changes in cholate content of bile, as well as in tauro/glyco acid ratio, were noted in the second series of experiments, with the exception of the 8th day of hypokinesia, when the concentration of glycocholic acids remained at the level of control values. In this experiment, tauro acid content was high (70% more than in the control).

Table 1. Intensity of bile secretion (mg/100 g weight/min) in hypokinetic white rats (M±m)

Experimental conditions	Number of experim.	Hours of experiment				Total bile in 4 h, g/kg	p
		1	2	3	4		
First series							
Background	17	4,3±0,3	4,1±0,3	3,9±0,3	4,2±0,3	9,90±0,46	
Hypokinesia, day:							
4	10	4,2±0,3	5,3±0,3	5,4±0,4	5,1±0,3	12,00±0,63	<0,002
8	8	6,4±0,2	5,8±0,3	4,7±0,3	4,3±0,2	12,72±0,40	<0,001
15	6	6,9±0,4	5,9±0,3	5,8±0,4	5,1±0,2	14,22±0,49	<0,001
30	9	4,8±0,2	4,3±0,2	4,5±0,4	3,8±0,2	10,44±0,66	>0,05
Second series							
Background	8	4,6±0,2	4,4±0,1	4,3±0,3	4,2±0,2	10,50±0,46	—
Hypokinesia, day:							
8	11	4,8±0,2	5,1±0,3	5,0±0,2	5,0±0,2	11,94±0,46	<0,05
15	11	4,8±0,3	4,5±0,3	4,8±0,2	4,7±0,2	11,28±0,46	0,25
30	12	5,3±0,2	5,3±0,3	4,8±0,3	4,7±0,2	12,06±0,39	<0,02

As was the case for bile acids, there was moderate increase in bile cholesterol concentration under hypokinetic conditions, particularly on the 4th and 8th days. Thereafter, this parameter declined. By the 30th day it reached the base level (second series) or was below it (first series). Because of the impaired concentrations of bile acids and cholesterol in bile, there was a change in cholate-cholesterol coefficient, which is an important indicator of stability of bile [2, 15]. Since the increment in bile acids in bile exceeded the increment in cholesterol, there was an increase of this coefficient. It is only on the 15th day of hypokinesia that it remained on the level of control values in the first series of experiments.

Less consistent changes were demonstrable in concentration of bilirubin in bile. In the first series, it either failed to change (8th day), or decreased (4th and 15th days), or else, on the contrary, increased (30th day) by about 1.5 times. In the second series of experiments, there was an increase in concentration of bilirubin in bile on the 8th and 15th days of hypokinesia, but some decrease on the 30th day.

Analysis of parameters of total cholates, bilirubin and cholesterol excreted in bile (Table 3) is very important to determination of the state of processes

of synthesis and secretion of bile acids and bilirubin, as well as excretion of cholesterol under hypokinetic conditions.

Table 2. Effect of hypokinesia on bile chemistry in white rats (M±m)

Indicators of bile chemistry	Back-ground	Day of hypokinesia			
		First series			
Total bile acid concentration, g/ℓ	10,53±0,95	16,17±1,75*	21,34±2,55*	10,54±0,60	17,65±0,
Breakdown:					
taurocholic acids	9,23±0,83	14,26±1,91*	15,38±2,30*	7,93±0,32	10,62±0
glycocholic acids	1,30±0,12	1,91±0,47	5,96±0,39*	2,61±0,30*	7,03±0,
Tauro-/glyco-acid ratio	7,1:1	7,5:1	2,6:1	3,0:1	1,5:1
Bilirubin concentration, μmol/ℓ	163±2,0	119±6,6*	159±15,3	113±11,8*	242±2
Cholesterol concentration, μmol/ℓ	487±14,1	521±11,3	583±47,8*	526±14,1	263±6
Cholate/cholesterol coefficient	57	80	101	53	174
		Second series			
Total bile acid concentration, g/ℓ	10,58±0,97	-	16,50±1,40*	13,19±1,05	13,94±0
Breakdown:					
taurocholic acids	8,70±1,03	-	14,83±1,36*	8,14±1,22	10,61±0
glycocholic acids	1,88±0,22	-	1,67±0,16	5,05±0,82*	3,33±0
Tauro-/glyco-acid ratio	4,6:1		8,8:1	1,6:1	3,2:1
Bilirubin concentration, μmol/ℓ	222±8,8	—	281±11,6*	358±61,6*	193±2
Cholesterol concentration, μmol/ℓ	414±21,8	—	497±33,2*	436±37,1	390±
Cholate/cholesterol coefficient	67	—	87	78	92

Note: Here and in Table 3, asterisks indicate reliable changes ($P<0.05$), as compared to background.

We found that total cholates in bile increased more under hypokinetic conditions than their concentration, due to increase in rate of bile secretion. Already on the 4th day, there was a 1.8-fold increase in this parameter, 2.7-fold increase on the 8th day, 1.5- and 1.7-fold increases on the 15th and 30th days, respectively (first series). The increment of total quantity of cholates occurred at the expense of taurocholic acids, the amounts of which increased by 1.8, 2.2, 1.2 and 1.2 times on the respective days of hypokinesia, while glycocholic acids increased by 2.0, 6.3, 3.3 and 5.7 times. Analogous changes were demonstrated in the second series.

As was the case with bile acids, there was increase in indicator of total amount of cholesterol excretion in bile per unit time: by an average of 25-50% in the first 4-15 days of hypokinesia. By the 30th day, cholesterol excretion decreased on the average from 0.12±0.005 to 0.07±0.005 μmol/ℓ/h (first series). In the second series, no decrease was demonstrable in excretion of this steroid

Table 3. Effect of hypokinesia on excretion of bile acids, bilirubin and cholesterol in bile of white rats (M±m)

Parameter	Background	Day of hypokinesia			
		4	8	15	30
First series					
TOTAL BILE ACIDS, G/KG/H	0.0026 ± 0.0027	0.048 ± 0.0029*	0.070 ± 0.0149*	0.038 ± 0.0463*	0.045 ± 0.0034*
TAURO ACIDS	0.023 ± 0.023	0.042 ± 0.0041*	0.051 ± 0.0078*	0.028 ± 0.0027	0.028 ± 0.0025
GLYCO ACIDS	0.003 ± 0.0005	0.006 ± 0.0014*	0.019 ± 0.0023*	0.010 ± 0.0021*	0.017 ± 0.0016*
TAURO/GLYCO ACID RATIO	7.7	7.0	2.7	2.8	1.6
TOTAL BILIRUBIN, μMOL/KG/H	0.11 ± 0.014	0.16 ± 0.030	0.52 ± 0.104	0.40 ± 0.024	0.60 ± 0.036*
TOTAL CHOLESTEROL, μMOL/KG/H	0.12 ± 0.005	0.15 ± 0.007*	0.18 ± 0.023*	0.14 ± 0.009*	0.07 ± 0.005*
Second series					
TOTAL BILE ACIDS, G/KG/H	0.026 ± 0.0032		0.049 ± 0.0039*	0.031 ± 0.0049*	0.041 ± 0.0023*
TAURO ACIDS	0.021 ± 0.0031		0.044 ± 0.0037*	0.023 ± 0.0032	0.033 ± 0.0023*
GLYCO ACIDS	0.005 ± 0.0006		0.005 ± 0.0006	0.012 ± 0.0039*	0.010 ± 0.0010*
TAURO/GLYCO ACID RATIO	4.6		8.8	1.9	3.1
TOTAL BILIRUBIN, μMOL/KG/H	0.08 ± 0.008		0.54 ± 0.041*	1.01 ± 0.173*	0.90 ± 0.076
TOTAL CHOLESTEROL, μMOL/KG/H	0.11 ± 0.003		0.14 ± 0.007*	0.12 ± 0.009	0.12 ± 0.009

in bile. In turn, bilirubin secretion was either unimpaired or somewhat increased during hypokinesia (see Table 3).

Consequently, the results of our experiments indicate that moderate hypersecretion of bile develops in hypokinetic rats, and it is more marked in the fall-winter period. During long-term restriction of motor activity, there is drastic increase in synthesis and secretion of bile acids, as a result of which their levels in bile rise. There is also an increase in cholesterol excretion.

Apparently, hypercholesterolemia as a result of impaired lipid and carbohydrate metabolism is the initial factor that leads to the above changes in biligenesis [10, 14, 23]. The body is relieved of cholesterol excess via different routes, including its use for synthesis of bile acids [14]. It is excreted in part in bile, which leads to hypercholesterolia. In addition, it should be borne in mind that cholesterol per se can influence synthesis of bile acids, since it is a corrective agent for activity of microsomal enzymes of hepatocytes, which regulate synthesis and biotransformation of bile acids [26].

Evidently, the adrenal cortex, the activity of which increases when motor activity is restricted for at least the first 2-3 weeks, plays some part in the mechanism of bile hypersecretion and more intensive synthesis of bile acids under hypokinetic conditions [12, 22, 24]. The studies of a number of authors [2, 8, 15, 25, 27] established that the adrenal cortex and its glucocorticoid hormones play an important role in neurohumoral mechanisms of regulating the biligenic process.

Still unclear is the reason for more energetic formation of glycocholic acids in hypokinetic rats. Perhaps, this is attributable to disturbances in amino acid metabolism in the liver [7], as a result of which there is facilitation

of utilization of glycine for their synthesis or, on the contrary, greater difficulty in utilization of taurine. It can also be assumed that, in view of metabolic impairment under hypokinetic conditions, there is a change in activity of transferase, which are involved in formation of taurocholic and glycocholic acids from cholyl-CoA and, accordingly, taurine and glycine [15]. Additional studies are needed to settle this question.

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EFFECT OF SHORT-TERM HYPOKINESIA ON RAT OPIOID SYSTEM REACTION

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 18, No 4, Jul-Aug 84 (manuscript received 29 Dec 82) pp 63-66

[Article by R. A. Tigranyan, O. P. Vakulina and L. F. Panchenko]

[English abstract from source] The content of methionine-enkephalin, leucine-enkephalin and β-endorphine was measured in various brain compartments (hypophysis, hypothalamus, midbrain, medulla oblongata, striatum), adrenals and plasma of the rats exposed to single and repeated immobilization. The reaction of the opiate systems to immobilization was very distinct in the emotiogenic brain structures (hypothalamus and midbrain) and hypophysis. The content of opiate-like peptides varied as a function of the immobilization time, with the most distinct changes occurring at the 150th minute. After daily immobilization repeated 40 times adaptation to the chronic stress-effect developed.

[Text] Endogenous opioid peptides (endorphins and enkephalins) are concentrated in brain structures that control motor, autonomic and emotional reactions in response to nociceptive and stress factors (hypothalamus, thalamus, periaqueductal gray matter, limbic system, gelatinous substance of the spinal cord) [15]. It is believed that opioids play a substantial part in regulating a number of physiological functions in the body: respiration, arterial pressure, function of endocrine and digestive systems, body temperature, as well as physiological response to extreme factors [2, 9]. Like morphine, opioids attenuate sensitivity to pain and, having a selective effect on the emotional component of pain perception, determine the psychoemotional state in stress situations [2, 9]. It should be noted that most studies of the role of opioids in the defense reaction to stress were conducted with use of behavioral tests, where involvement of opiate systems in the reaction to stress was confirmed by administration of naloxone; the biochemical data are sparse and contradictory. It was shown that rat blood plasma β-endorphin (BE) level rose drastically under the effect of electric current [10, 13], swimming in cold water [17] and immobilization [11]. According to several authors [8, 18], elevation of enkephalin levels was noted in brain tissues after stress, whereas other authors [4, 10, 12, 13, 17] observed a decrease in concentration of enkephalins and BE in the hypothalamus and stability of their levels in other parts of the brain. On the assumption that one of the causes of the above

contradictions is that the authors used stress stimuli differing in intensity, we made a study of levels of opioid peptides in rat tissues and blood during different forms of immobilization lasting for different periods of time.

Methods

We conducted our study using male Wistar rats weighing 250-300 g. Immobilization was produced by securing the animals on special tables by the Selye method [7]. They were tested following single immobilization lasting 5, 30 and 150 min, as well as repeated immobilization (150 min daily for 7 and 40 days). In view of the fact that the response of opiate systems to stress is a function of time of day [18], the rats were decapitated at the same times. We assayed methionine-enkephalin (ME), leucine-enkephalin (LE) and BE in different parts of the brain (hypophysis, hypothalamus, midbrain, medulla oblongata and striatum), ME in the adrenals and BE in blood plasma. Opioids were assayed by the method of radioimmune analysis (RIA) using commercial sets of the Immunonuclear Corporation (United States).

Preparation of brain tissues and adrenals for analysis consisted of acid extraction in 1 M CH_3COOH (2 mℓ per sample) for 15 min in a boiling water bath, followed by homogenization of the cooled specimen and centrifugation of homogenate at 6000 G for 15 min at 6°C. The precipitate was again homogenized in 1 M CH_3COOH and centrifuged under the same conditions; both supernatants were decanted and neutralized with NaOH. For analysis of brain tissue samples, we took 0.5 mℓ supernatant to assay enkephalins and stored it at -20°C; the rest was lyophilized. Supernatants of adrenal samples were lyophilized after neutralization, and prior to assaying ME they were diluted in RIA buffer to the concentration required for analysis. Lyophilisates of brain tissues were dissolved in 0.7 mℓ 0.1 M borate buffer containing 0.1% ox serum albumin (pH 8.4) to separate BE from β-lipotropic hormone; after centrifugation of the samples at 6000 G for 5 min, the samples were submitted to gel filtration on a 0.9×25 cm column packed with Sephadex G-50 in the same buffer at 4°C; fractions containing BE were combined and used for analysis. We added 850 μg/mℓ bacitracin to the plastic tubes, in which blood was collected, to prevent enzymatic proteolysis of blood BE. An aliquot of plasma (0.5 mℓ) was submitted to gel filtration under conditions analogous to those described above for BE extraction from brain tissue.

Statistical reliability was calculated with use of Student's t test.

Results and Discussion

Immobilization of rats for 5 min elicited significant increase in concentrations of LE and BE in the hypophysis and mesencephalon, as well as of LE in the striatum (Table 1); no changes were found in ME level, which is indicative of differences in reactions of leu- and met-enkephalinergic systems to a brief stressor.

Rat immobilization for 30 min led to decline of the elevated opioid levels (demonstrated in the 5th min of immobilization) in the midbrain, hypophysis and striatum, as well as significant decrease in enkephalin content of the hypothalamus (see Table 1). The decline of enkephalin levels in the hypothalamus, which was observed by a number of researchers with other types of stress

[4, 10, 12], was apparently due to more intensive utilization of these peptides in this brain structure. At the same time, we failed to demonstrate a decrease in BE concentration in the hypothalamus after 30-min exposure to stress, which had been shown by a number of authors [10, 13, 17]. Moreover, we did not observe the drastic increase in blood BE content, which had been demonstrated by several authors [10, 11, 13, 17] with different forms of stress.

Table 1. Concentrations of opioid peptides in the brain (pmol/mg), adrenals (pmol/mg) and blood (fmol/mℓ) of rats (M±m; n = 6)

STRUCTURE	OPIOID PEPTIDE	SINGLE IMMOBILIZATION MIN					
		CONTROL	EXPERIM.	CONTROL	EXPERIM.	CONTROL	EXPERIM.
STRIATUM	ME	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	LE	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
HYPOTHALAMUS	ME	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	LE	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	BE	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
HYPOPHYSIS	ME	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	LE	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	BE	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
MESENCEPHALON	ME	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	LE	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	BE	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
MEDULLA OBLONGATA	ME	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	LE	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	BE	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
ADRENALS	ME	0.133±0.007	0.188±0.031	0.076±0.012	0.061±0.002	0.034±0.003	0.040±0.[illegible]
	BE	0.0060±0.0009	0.0068±0.0003	0.0116±0.0043	0.0104±0.0018	0.010±0.001	0.008±0.001
BLOOD PLASMA	ME	0.022±0.003	0.017±0.002	0.023±0.008	0.022±0.004	0.020±0.002	0.027±0.[illegible]
	BE	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Note: Here and in Table 2, *P<0.05, **P<0.02, ***P<0.01.

Table 2. Concentration of opioid peptides in the brain (pmol/mg), adrenals (pmol/mg) and blood (fmol/mg) of rats (M±m; n = 6)

STRUCTURE	OPIOID PEPTIDE	REPEATED IMMOBILIZATION			
		7 · 150 MIN		40 · 150 MIN	
		CONTROL	EXPERIMENT	CONTROL	EXPERIMENT
STRIATUM	ME	1.05±0.12	1.34±0.[illegible]	[illegible]±0.23	1.50±0.24
	LE	0.522±0.[illegible]	0.526±0.[illegible]***	[illegible]±0.028	0.[illegible]±0.085
HYPOTHALAMUS	ME	0.78±0.034	0.85±0.1[illegible]	[illegible]±0.20	0.93±0.[illegible]
	LE	0.345±0.041	0.388±0.[illegible]	0.345±0.022	0.322±0.013
	BE	0.121±0.020	0.094±0.024	0.130±0.030	0.116±0.012
HYPOPHYSIS	ME	0.398±0.034	0.840±0.031	0.85±0.25	2.42±0.55*
	LE	0.[illegible]±0.024	0.[illegible]±0.[illegible]	[illegible]±0.008	0.439±0.[illegible]
	BE	0.77±0.[illegible]	[illegible]±0.20	[illegible]±0.20	[illegible]±0.[illegible]
MESENCEPHALON	ME	0.211±0.032	0.251±0.[illegible]	0.349±0.009	[illegible]±0.[illegible]
	LE	0.086±0.[illegible]	0.120±0.[illegible]***	0.085±0.004	0.082±0.[illegible]
	BE	0.0082±0.0009	0.010±0.002	0.012±0.002	0.014±0.[illegible]
MEDULLA OBLONGATA	ME	0.234±0.025	0.236±0.[illegible]	[illegible]±0.[illegible]	0.46±0.045
	LE	0.[illegible]±0.[illegible]	0.452±0.[illegible]	0.076±0.012	0.[illegible]±0.[illegible]
	BE	[illegible]±0.[illegible]	[illegible]±[illegible]	[illegible]±0.[illegible]	[illegible]±0.[illegible]
ADRENALS	ME	0.022±0.003	0.011±0.001**	0.023±0.008	[illegible]±0.[illegible]
BLOOD PLASMA	BE	158.2±24.2	323.1±98.[illegible]	[illegible]±[illegible]	[illegible]±29.2

With longer immobilization (150 min), there was no further decline of enkephalin levels, on the contrary, we observed a drastic increase in their concentration in the hypothalamus and hypophysis (see Table 1). It is known that hypophyseal opioids play an important part in forming the behavior of rats under stress [1]. It should be noted that we demonstrated for the first time in our study the involvement of the enkephalinergic system of the hypophysis in the stress reaction. Hypophyseal BE level dropped to 5/13ths (of control), whereas in blood plasma it increased by 2 times (see Table 1), which suggests that there is discharge of BE from the hypophysis into systemic circulation.

The decrease in hypophyseal BE concentration under stress, which is associated with elevation of its level in blood plasma, was observed by many authors [3, 10, 14]. Adrenocorticotropic hormone (ACTH) and BE are secreted simultaneously in blood under stress, and in equimolar amounts [5]. Perhaps, ACTH determines the metabolic component of the adaptation reaction and BE its psychogenic component. The increase in hypothalamic BE content and decrease in the hypophysis confirm the assumption that there is retrograde efflux of BE from the hypophysis into the hypothalamus [6]; however, we cannot rule out the possibility of BE synthesis from β-lipotropin in the hypothalamus itself [16].

When rats were immobilized daily for 1 week, there was incrase in LE content of the striatum and midbrain, as well as decreased to one-half of ME level in the adrenals (Table 2). It is known, that enkephalinoid material is localized in the adrenals in the axon endings of splanchnic nerves and chromaffin cells, where it is in granules together with catecholamines. Stimulation of catecholamine synthesis and release in vitro elicits cosecretion of opioids from the chromaffin granules and increase in their synthesis [19]. The absence of changes in adrenal ME level after acute stress is apparently related to the fact that the more intensive discharge of opioids was counterbalanced by simultaneous increase in their synthesis (the RIA method we used also demonstrated higher molecular precursors of ME). By the 7th day of immobilization there was already appreciable decline of ME level in the adrenals. This indicates that immobilization repeated 7 times was not associated with adaptation of rats to the stressor.

After 40-fold daily immobilization, we failed to demonstrate reliable changes in opioid levels in tissues of the brain, adrenals and blood (with the exception of unexplainable increase in ME concentration in the hypophysis) (see Table 2), which is indicative of development of adaptation to the chronic stress factor we used.

Thus, the response of opiate systems to immobilization stress was particularly marked in emotiogenic structures of the brain (hypothalamus and mesencephalon) and hypophysis. Evidently, the function of the hypothalamus-hypophysis-adrenal axis is under the control of opioids. The levels of opioid peptides in brain tissues changed as a function of duration of immobilization. The demonstrated three-phase nature of fluctuations in opioid levels is indicative of adaptive changes in the opiate system in the course of exposure to an acute stressor. The marked activation of the opioid system, which we demonstrated with longer (150 min) exposure to the stressor is indicative of its appreciable role in protecting the body against injury during prolonged exposure to the

stressogenic factor. Our findings are indicative of the regulatory role of opioid peptides when the body is submitted to hypokinesia, which is one of the extreme factors of spaceflights.

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REACTION OF CEREBRAL VENTRICLES TO ANTIORTHOSTATIC POSITION AND OCCLUSION OF JUGULAR VEINS

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 18, No 4, Jul-Aug 84 (manuscript received 11 Apr 83) pp 66-69

[Article by V. I. Sokolov]

[English abstract from source] Twenty test subjects were subdivided into two groups with respect to the reactions of brain ventricles to a preliminary head-down test (-30°). Group 1 consisted of subjects who showed ventricular enlargement, headache and nausea; group 2 included subjects without these reactions. The test subjects were also exposed to tests with neck vein occlusion. The first (continuous) test was performed at +40 mm Hg for 5 min; the second (step) test was carried out from +10 to +50 mm Hg for 2 min at each step. The results obtained demonstrate that an adequate venous outflow from the cranial cavity influences brain ventricles; the data also give evidence that changes in compensatory mechanisms can be detected using occlusion tests.

[Text] In the practice of expert professional certification it is particularly important to develop methods of evaluating compensatory and adaptive mechanisms of the system of cerebral circulation of blood and spinal fluid. Studies conducted on animals [3, 4], as well as with the participation of man [5, 6], revealed that, during use of the antiorthostatic [head-down tilt] factor, which is a model of redistribution of body fluids in a cranial direction, there is elevation of intracranial spinal fluid pressure, which could lead to a decrease in cerebral blood flow and development of circulatory hypoxia [10]. The close link between spinal fluid and venous pressure in the system of cerebral circulation of blood [3] makes it necessary to investigate the compensatory mechanisms of delivery of spinal fluid to the cranial cavity with consideration of the nature of efflux of venous blood. Impaired venous efflux in the jugular veins is one of the basic pathogenetic mechanisms in development of intracranial spinal fluid hypertension [8]. All this prompted us to investigate the reactions of the cerebral ventricular system, which provide an indirect idea about the dynamics of intracranial pressure of cerebrospiral fluid [7].

Methods

Our investigation involved the participation of 20 male subjects 26 to 35 years of age.

We examined the ventricular system of the brain by means of one-dimensional echoventriculometry [6]. We calculated the ventricular indexes: index of the third ventricle (Dv_i), which equals the ratio of diameter of the head (doubled transmission) to size of the third ventricle, and index of the medial wall of the lateral ventricle (Pm_i), which is the ratio of magnitude of transmission to distance between the medial wall of the lateral ventricle and third ventricle. The ventricular indexes reflect changes in correlation between volumes of ventricles and cerebral tissue as a result of change in cerebrospinal fluid pressure in the cranial cavity [1, 9].

Ultrasonic probing was performed in the emission mode at a frequency of 0.88 MHz in the central temporal lead. We used an EES-12 echoencephaloscope manufactured in the USSR.

The cervical veins were occluded by two methods. The first (simple occlusion test) consisted of continuous occlusion of veins of the neck, including the jugular veins, for 5 min in the mode of +40 mm Hg occlusion, and the second (stepped occlusion test), of occlusion of cervical veins in modes of +10 to +50 mm Hg. Each occlusion step had a value of 10 mm Hg and lasted 2 min, with 1-min intervals between steps.

The results were submitted to statistical processing with consideration of Student's criterion ($P<0.05$).

Results and Discussion

All of the subjects were divided into two groups. This was done on the basis of the two types of reactions of cerebral ventricular system observed in a preliminary antiorthostatic test (-30°) for 20 min, which causes redistribution of body fluids in a cranial direction, which is one of the pathogenetic factors in the effects of weightlessness on man. The first group consisted of men who presented dilatation of the cerebral ventricular system in response to head-down tilt, which was indicative of elevation of intracranial spinal fluid pressure. This was associated with appearance of general cerebral symptoms, i.e., sensation of blood rushing to the head and heaviness of the head, changing to a splitting headache, slight nausea (with emesis in two cases), illusion of rocking, etc. (10 subjects). The second group consisted of individuals who did not present signs of dilatation of the ventricular system of the brain or the above-described clinical symptoms (10 men).

Table 1 lists the results of testing reactions of the ventricular system of the brain with the simple occlusion test.

We found that there were no reliable changes in the index of the third ventricle (Dv_i) in the second group of subjects during 5-min occlusion ($P>0.05$). This was indicative of good compensatory capacities of the system of spinal fluid circulation in this group, probably mainly due to inclusion of a retro-

retromastoid anastomosis [10] and discharge of venous blood into the venous plexus of the spine (negative Queckenstedt sign). In the first group of subjects, there was an increase in Dv_i in the 1st min, which was indicative of compensatory decline of spinal fluid pressure in response to increased venous filling of the cranial cavity [3]. It must be noted that signs of dilatation of lateral ventricles of the brain were not present in either group of subjects in the background test (Pm_i was not determined). By the end of the 3d min, the 1st group of subjects presented dilatation of lateral ventricles without reliable changes in the third ventricle (Dv_i did not differ reliably from the base level). It can be assumed that the dilatation of lateral ventricles is related to increase in spinal fluid pressure due to increased secretion and decreased resorption of spinal fluid as a result of poorer venous efflux from the cranial cavity. By the 5th min of occlusion, there was a 50.8% decrease in Dv_i, as compared to the base value, while Pm_i did not differ reliably from the value in the 3d min of occlusion. Such changes were associated with the sensation of heaviness and flushing of the head, appearance of microphotopsy, which was inherent in these subjects when the preliminary antiorthostatic test was performed (positive Queckenstedt sign). After discontinuing the simple occlusion test, the 1st group of subjects presented a 28.3% increase in Dv_i, as compared to the base level, which was indicative of a drop in intracranial pressure of spinal fluid.

Table 1. Dynamics of index of third ventricle (Dv_i) and index of medial wall of lateral ventricle (Pm_i) in 1st and 2d groups of subjects during simple occlusion test (occlusion mode +40 mm Hg)

Group of subjects	Parameter	Occlusion time, min: backgr.		1		2		3		4		5	
		Dv_i	Pm_i	Dv_i	Pm_i	Dv_i	Pm_i	Dv_i	Pm_i	Dv_i	Pm_i	Dv_i	Pm_i
1	*M*	20,5	—	27,0	—	18,3	—	18,8	7,8	13,3	5,4	13,6	5,6
	m	1,3	—	2,1	—	2,4	—	1,7	1,7	1,7	0,4	1,6	0,8
	P	—	—	<0,05	—	>0,05	—	>0,05	—	<0,01	>0,05	<0,01	>0,05
2	*M*	26,4	—	26,7	—	29,2	—	28,8	—	26,9	—	26,7	—
	m	1,3	—	2,1	—	1,7	—	1,4	—	1,8	—	1,3	—
	P	—	—	>0,05	—	>0,05	—	>0,05	—	>0,05	—	>0,05	—
	$P_{1,2}$	<0,01	—	>0,05	—	<0,01	—	<0,01	—	<0,001	—	<0,001	—

We used the stepped occlusion test to demonstrate that changes in the cerebral ventricular system is a function of degree of difficulty in venous efflux from the cranial cavity in the system of jugular veins. The results of these tests are listed in Table 2.

The 2d group of subjects presented a reliable fall of spinal fluid pressure, which was manifested by constriction of the third ventricle (Dv_i increased by 41.6%, in comparison to base value) with occlusion in modes of +30 and +40 mm Hg. There were no signs of dilatation of lateral ventricles (Pm_i was not determined). However, with +50 mm Hg occlusion, there was moderate increase in the index of the medial wall of the lateral ventricle, which was indicative of dilatation of the lateral ventricle and elevation of spinal fluid pressure, probably as a result of disruption of compensatory mechanisms of spinal fluid circulation.

Table 2. Dynamcis of index of third ventricle (Dv_1) and index of medial wall of lateral ventricle (Pm_1) in 1st and 2d groups of subjects during stepped occlusion test

Occlusion mode, mm Hg	Occlusion time, min	Group of subjects							
		1				2			
		Dv_1		Pm_1		Dv_1		Pm_1	
		M ± m	P	M ± m	P	M ± m	P	M ± m	P
	Back-ground	20,7 ± 1,1				29,6± 1,1			
10	1	24,1 ± 0,9	>0,05			27,5± 1,2	>0,05		
	2	20,9 ± 1,0	< 0,05			27,6± 0,9	>0,05		
20	1	21,7 ± 1,6	>0,05	6,8± 1,1		26,5± 1,8	>0,05		
	2	17,6 ± 1,1	< 0,01	5,9± 0,8	>0,05	26,8± 1,1	>0,05		
30	1	18,8 ± 1,3	>0,05	7,8± 1,2	>0,05	34,0± 1,2	<0,01		
	2	17,9± 1,2	<0,05	7,2± 0,9	>0,05	34,0± 1,7	<0,01		
40	1	18,4± 2,3	>0,05	10,1± 0,9	<0,001	39,1± 1,9	<0,01		
	2	16,6± 1,4	<0,01	10,1± 0,9	<0,001	39,1± 0,8	<0,01		
50	1	18,0± 1,1	>0,05	10,3± 0,8	<0,001	23,8± 1,8	<0,05	4,2± 1,1	
	2	16,4± 1,1	<0,01	1 .2± 0,9	<0,001	20,7± 1,9	<0,01	6,9± 0,7	>0.05

The 1st group of subjects showed dilatation of lateral ventricles in the 1st min of occlusion of +20 mm Hg, and by the 2d min there was a reliable decline of index for the third ventricle (Dv_1) by 11.8% in comparison to base value. Such changes are indicative of elevation of spinal fluid pressure in the cavity of the third ventricle and its dilatation [1]. Thereafter, we observed increase of Pm_1 and decrease of Dv_1. Dilatation of the cerebral system of ventricles reached a maximum at +50 mm Hg occlusion. These changes were associated with intensification of clinical symptoms (appearance of headache, nausea, etc.). Such differences in reactions of the cerebral ventricular system in the 1st and 2d groups of subjects could be attributed to differences in degree of development of collateral circulation and arteriovenous anastomoses, on the one hand, and differences in venous resistance, on the other.

Thus, the reactions of the ventricular system of the brain have a close functional link with the degree of difficulty in venous efflux from the cranial cavity. The occlusion tests we used could serve to assess flexibility and adequacy of compensatory and adaptive mechanisms of spinal fluid and blood circulation in the brain.

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UDC: 612.262-06:612.766.1

EFFECT OF MAXIMUM PHYSICAL LOAD ON OXYGEN-TRANSPORT PROPERTIES OF BLOOD

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 18, No 4, Jul-Aug 84 (manuscript received 21 Apr 83) pp 69-72

[Article by L. A. Ivanov and N. D. Chebotarev]

[English abstract from source] Ventilation, gas exchange, gas composition and pH of venous blood, as well as oxyhemoglobin dissociation curves were investigated during exercise tests of 9 healthy volunteers aged 19-31. During maximal exercises the dissociation curve shifted to the right. The shift was associated with the extraerythrocyte factor, i.e. the Bohr effect due to metabolic acidosis in muscles. The shift which indicates a lower hemoglobin affinity for oxygen and a higher oxygen release by blood is of adaptive importance: during exercises oxygen supply to tissues increases. This shift is also evidenced by an increase of venous pO_2 during muscle work.

[Text] Muscular activity is viewed, with complete justification, as the most powerful and multifaceted stimulator of autonomic functions. During physical labor there is a many-fold increase in metabolic processes in working muscles, which puts high demands on the "service," in the definition of G. V. Fol'bort [5], systems--respiratory and cardiovascular. Indeed, a comparison of the data of A. Z. Kolchinskaya [3] concerning reactions of adolescents 15-16 years of age to a maximum physical load and marked hypoxia (11.6% oxygen in inhaled air) revealed that the heart rate, respiration rate and tidal volume, minute volume of blood, minute respiratory volume, oxygen pulse and oxygen uptake during physical exercise exceeded the levels during hypoxia by 2.4, 3, 4, 9, 16 and 17 times, respectively. For this reason, the physical load test has found broad application in aviation and space medicine.

The blood system is important to delivery of oxygen to tissues during muscular activity. Yet the respiratory function of blood during muscular activity has not been sufficiently investigated. Our objective here was to study the oxygen-transport function of blood with a maximum physical load.

Methods

We examined 9 essentially healthy men 19-31 years of age. We used a stepped physical load, which was increased to the maximum level, in the form of

vertical pedaling on a cycle ergometer at the rate of 50 r/min. The initial load and increment at each step constituted 25 W. Each step lasted 5 min. We recorded on an energy analyzer the parameters of ventilation and exchange of gases during exericse.

In the background state and 4th-5th min of the last step of the load, we drew blood from the ulnar vein for measurement of pO_2, pCO_2, pH and to plot the curve of oxyhemoglobin dissocation. We found the pH electrochemically and pO_2 by the polarographic method using a Clark electrode. Measurements were made on a Microastrum instrument (Radiometer Firm, Denmark). We calculated pCO_2 on the basis of parameters of acid-base equilibrium.

The oxyhemoglobin dissociation curve which was plotted with a DSA-1 instrument (Radiometer Firm, Denmark), was analyzed.

The methods for measuring gases, blood pH and analysis of the oxyhemoglobin dissociation curve have been described in detail previously [2].

Results and Discussion

As shown by our studies, there was distinct elevation of pO_2 in the subjects' unadulterated blood, corresponding to the levels of oxygenation of hemoglobin (40-80%) at which blood comes in contact with tissues (Table 1). This is indicative of a right shift of the oxyhemoglobin dissociation curve (Figure 1) and better delivery of oxygen to tissues.

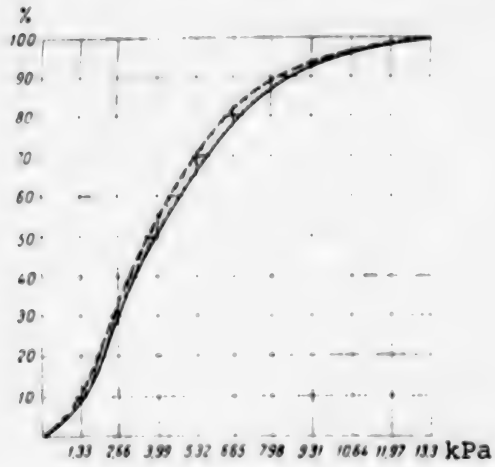

Figure 1.
Curve of dissociation of hemoglobin in native blood in background state (dash line) and with maximum physical load (solid line).
Here and in Figure 2:
x-axis, blood plasma oxygen tension (in kPa); y-axis, oxygenation of hemoglobin (%)

Table 1.
Change in parameters of oxyhemoglobin dissociation curve (in kPa) with maximum physical load, as compared to base levels

Parameter	Native blood oxyhemoglob. dissociation curve		Standard oxyhemogl. dissociation curve	
	shift	P	shift	P
P_{10}	0,089	>0,05	-0,076	>0,05
P_{20}	0,089	>0,05	-0,172	<0,01
P_{30}	0,104	>0,05	-0,227	<0,01
P_{40}	0,208	<0,05	-0,187	<0,05
P_{50}	0,267	<0,01	-0,176	<0,05
P_{60}	0,355	<0,01	-0,267	<0,01
P_{70}	0,341	<0,05	-0,341	<0,01
P_{80}	0,371	<0,05	-0,480	<0,01
P_{90}	0 280	>0,05	-0,685	<0,01
P_{95}	0,267	>0,05	-0,870	<0,01
P_{100}	-0,280	>0,05	-1,706	<0,01

This finding is consistent with data in the literature. Thus, in a study of athletes in different fields using polarographic coulometry, an increase was demonstrated in rate of oxygen discharge by blood during intensive muscular activity [6]. Taunton et al. [19] discovered a 2.8 mm Hg increase in P_{50} in 10 men after exercising until they were tired. An increase in P_{50} after a load to tiredness was demonstrated by Klein et al. [16] and with a submaximum load by Bonsignore et al. [10].

Studies of standard, i.e., adjusted to pH 7.4, curves of oxyhemoglobin dissociation revealed just the opposite tendency, i.e., there was a decline in values of pO_2 corresponding to all examined levels of hemoglobin oxygenation (see Table 1). This reflects an increase in affinity of hemoglobin for oxygen with a left shift of the curve of oxyhemoglobin dissociation (Figure 2).

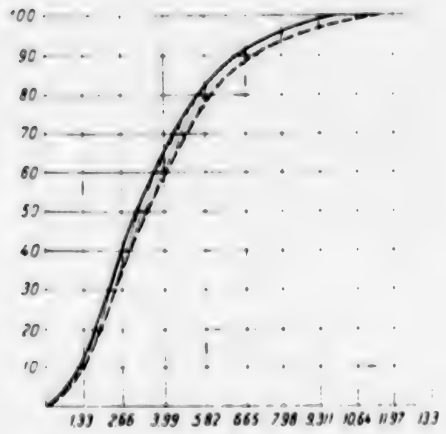

Figure 2.
Standard curve of oxyhemoglobin dissociation in background state (dash line) and with maximum physical load (solid line)

Table 2.
Parameters of gas composition (kPa) and pH of venous blood during maximum physical load

Parameter	M±m	Change in comparison to base values
pO_2	7,57±0,56	3,16*
pCO_2	4,80±0,19	−1,27*
pH	7,257±0,0024	−0,073*

*$P<0,01$.

Thus, with maximum physical load there is a contradiction between the increased affinity of hemoglobin for oxygen under standard conditions and decrease of this parameter in unadulterated blood. Apparently, factors appear in blood with a maximum physical load that are instrumental in the right shift of the curve of oxyhemoglobin dissociation, in spite of the increase in affinity of hemoglobin for oxygen.

Indeed, there was change in parameters of gas composition and pH of blood, with decline of p_vCO_2 (Table 2). This is apparently attributable to hyperventilation during maximum physical load, when minute volume reached 91.3±6.22 ℓ with output of carbon dioxide exceeding the base level by 14 times. Since venous blood was drawn from the nonfunctioning extremity (arm), the decline of p_aCO_2, which was due to increased elimination of CO_2, was not compensated by increased access of CO_2 from tissues, which is what led to decline of p_vCO_2 and, consequently, development of respiratory alkalosis. In addition, there is, as we know, accumulation of blood lactate due to glycolytic processes during intensive exercise, which are triggered when there is inadequate delivery of oxygen to working muscles. This is manifested by greater output of carbon dioxide (3607±294 mℓ), as compared to oxygen uptake (3243±208 mℓ), with rise in respiratory quotient to 1.12±0.079

(base value 0,87±0.032), since the increase in carbon dioxide output, in relation to oxygen uptake, with a physical load is due to expulsion of CO_2 from blood bicarbonates by nonvolatile acids (lactate).

In other words, the parameters of acid-base state of venous blood were found to be under the influence of opposite factors during muscular activity--increased CO_2 output due to hyperventilation and increased access of incompletely oxidized metabolic products from working muscles. There was prevalence of influence of metabolic acidosis, which was manifested by a decline of venous blood pH (see Table 2), which caused decrease in affinity of hemoglobin for oxygen. Consequently, the right shift of the curve of oxyhemoglobin dissociation with a physical load was related to an extraerythrocytic factor, decline of blood pH due to metabolic acidosis.

As for the opposite tendency, the left shift of the curve of oxyhemoglobin dissociation under standard conditions, the following should be taken into consideration. Metabolic acidosis leads to decrease in 2,3-diphosphoglycerate (2,3-DPG) content of erythrocytes [10, 13], the concentration of which is, as we know, inversely proportional to hemoglobin affinity for oxygen.

It would seem that this is in contradiction with data in the literature concerning increase in 2,3-DPG concentration during muscular activity [7, 14, 19]. Analysis of data in the literature indicates, however, that, in the first place erythrocyte 2,3-DPG content increases only during short-term exercise [7, 14]. It is expressly with this type of load that the greatest changes are observed in deoxygenated hemoglobin, catecholamines and plasma phosphates [8, 19], i.e., factors that influence 2,3-DPG concentration in red blood cells. At the same time, when exercising until fatigue sets in, 2,3-DPG content of erythrocytes not only failed to increase [9, 11, 20], but decreased [10]. In the second place, a significant part of the authors who reported increase in 2,3-DPG during exercise assayed it after the load. Yet it has been established that it is expressly in the postload period that the concentration of 2,3-DPG increases [16]. In the third place, it was demonstrated that 50-80% variability of hemoglobin affinity for oxygen during muscular activity is attributable to other factors, rather than changes in 2,3-DPG [16].

Thus, with maximum physical load the curve of oxyhemoglobin dissociation is shifted to the right, and this is due to the effect of metabolic acidosis (Bohr effect). This shift is undoubtedly of positive value, in the sense of adequate delivery of oxygen to tissues. The fact of the matter is that, during muscular activity, in spite of intensive function of autonomic systems, there is poorer delivery of oxygen to working muscles. This is manifested by such well-known phenomena of muscular activity as increase in blood lactate and increased oxygen uptake in the postexercise period--oxygen debt. There is also indirect evidence of hypoxia of functioning muscles, such as drastic decline of oxygenation of venous blood flowing away from the working limb [15, 17, 18].

For this reason, the terms, "motor hypoxia" [1] and "load hypoxia" [4], which are used to characterize oxygen conditions during muscular activity, are considered to be quite justified.

In the above situation, the right shifts of the curve of oxyhemoglobin dissociation, along with adaptive reactions of other physiological systems involved in gas exchange, are instrumental in improving delivery of oxygen to tissues. The rise of p_vO_2 (see Table 2) is indicative of the efficacy of this mechanism, since p_vO_2 reflects tissular pO_2 [12].

Thus, in the subjects we examined during maximum physical exercise, there was a right shift of the curve of oxyhemoglobin dissociation in native blood. This shift is due to the Bohr effect as a result of metabolic acidosis, which develops during physical exercise and improves delivery of oxygen to tissues. The rise of venous blood pO_2 during muscular activity is indicative of the efficacy of this mechanism.

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EFFECT OF HYPEROXIA AND INCREASED GAS ATMOSPHERE DENSITY ON CONTRACTILE FUNCTION OF THE RIGHT VENTRICLE

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 18, No 4, Jul-Aug 84 (manuscript received 11 May 83) pp 73-76

[Article by V. I. Kuleshov and Yu. V. Namlinskiy]

[English abstract from source] Rheocardiography, phonocradiography and electrocardiography were used to investigate right ventricle function of 16 test subjects exposed to various gas atmospheres under increased pressure. During short-term exposures the adverse effect of an increased pressure on the contractile function of the right ventricle grew in the following order: hyperoxia--normoxic nitrogen-oxygen atmosphere--compressed air.

[Text] Man's exposure to elevated atmospheric pressure (HP) is associated with a set of specific reactions by the cardiovascular system (CVS) [1, 5, 14]. The functional changes observed after single exposure to HP in function of the circulatory system during regular professional work under these conditions do not preclude onset and development of persistent premorbid states [1, 18] and a high level of CVS morbidity [6, 19] related to overloads on the right heart [1, 7, 19]. In spite of the extensive data pertaining to studies of the CVS with exposure to HP myocardial contractile function (MCF) under these conditions has been little-studied. Yet investigation of MCF during exposure to HP would enable us to gain deeper understanding of the mechanisms of development of adverse changes in CVS function. Our objective here was to offer a comparative description of contractile function of the right ventricle with separate and combined exposure to HP--hyperoxia and increased density of the gas environment.

Methods

This study involved the participation of 16 essentially healthy male volunteers 19 to 26 years of age. During the period of the study, the subjects were given the usual three meals, but were not allowed to take medication.

We used rheocardiography of the right ventricle [15]. We recorded the volumetric and differential rheogram (RG) of the pulmonary artery, phonocardiogram of the region of the apex beat (Botkin point) and ECG in the second standard lead. The RG was recorded using a 4-RG-02 rheograph. The equipment was in operation

throughout the period of investigation. Records were made at a tape-feed rate of 50 and 100 mm/s. We recorded at least 20 cardiac cycles on each subject. The recording unit of a physiograph (model 068) was used to record the rheocardiogram (RCG). Analysis of the RCG included determination of duration of cardiac cycle (CC), presphygmic (PS) and ejection (EP) periods, phases of isometric contraction (IC), rapid and slow ejection (RE and SE) by the method in [4], mechanical and overall systole (S_m and S_o), diastole (D_o), index of myocardial contraction (IMC), rheographic systolic index (RSI), intrasystolic coefficient (ISC), mechanical coefficient (MC), hemodynamic index (HI) and ejection coefficient (EC). EP, S_o and D_o were compared to the nominal values calculated using the formulas given in [10]. In addition, we made a loop analysis of the volumetric RG of the pulmonary artery, with consideration of its amplitude and morphological changes. Determination was made of the rheographic diastolic index (RDI) [13], peripheral resistance index (PRI), as well as maximum velocity of rapid filling (MVRF) [9, 13].

We conducted three series of studies. The background (control) study was performed with the subjects at relative rest (supine position), at normal pressure and with respiration of air (pO_2 = 0.021 MPa; density q = 1.16 g/ℓ).

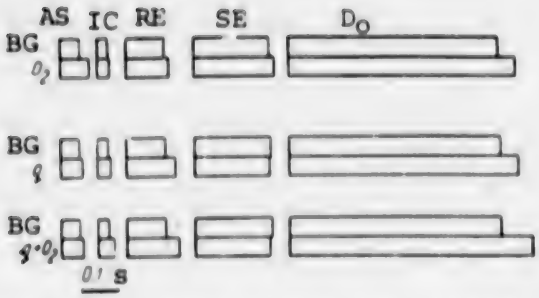
The first series of studies was conducted at normal pressure with the subjects breathing medical oxygen (pO_2 - 0.1 MPa, which corresponds to pO_2 when breathing air under pressure of 0.5 MPa). This virtually ruled out the factor of increased density of the gas atmosphere (q = 1.28).

The second and third series of studies were conducted in a continuous-flow decompression chamber (PDK-2) at 0.5 MPa pressure. The RCG was recorded by remote control (through leads into the pressure chamber) from the 30th to 35th min of exposure to high pressure. Decompression was performed in accordance with the working modes in "Rules for the Diving Service." In the second series, a compressed nitrogen-oxygen gas mixture was used for breathing (P = 0.5 MPa, q = 5.65 g/ℓ), the partial oxygen pressure in which was the same as in atmospheric air at sea level (0.021 MPa). In the third series, the same subjects breathed air while exposed to the combination of hyperoxia and high density of the gas environment (P = 0.5 MPa, pO_2 = 0.1 MPa, q = 5.81 g/ℓ). The temperature of the breathing mixtures was 20-22°C and pCO_2 up to 4 mm Hg.

The obtained data were processed using Student's t criterion ($P<0.05$).

Results and Discussion

When breathing medical oxygen in the first series, the subjects presented the following reliable ($P<0.05$) functional changes in the right ventricle (see Figure and Table): extension of CC, IC, EP, RE, S_o, D_o, with tendency toward extension of AS [arterial systole] and SE; there was increase in IMC, HI and decrease in EC; ISC showed virtually no change. Such changes could be indicative of increased load of elastic resistance and appearance of high diastolic pressure syndrome [11]. In the second series, with increased gas atmosphere density under normoxic conditions (pO_2 = 0.021 MPa), there were changes in CC structure that differed from the changes occurring in the first series. There was increase in duration ($P<0.05$) of CC, IC, PS, RE, EP, S_o, D_o, with a tendency toward increase in AS; SE time, ISC and MC decreased, while HI and



Phase structure of right ventricle function

BG) background (results of control study)
O_2) effect of oxygen alone
q) effect of high density alone (normoxic nitrogen-oxygen mixture, breathing under elevated pressure)
$q+O_2$) combined effect of increased density and hyperoxia (atmospheric air, breathing under elevated pressure)

and EC increased, which is typical of the syndrome of functional myocardial hypodynamia [10]. In the third series, where the subjects breathed atmospheric air under pressure of 0.5 MPa, the changes in the parameters examined presented the same orientation as in the second series, but they were more marked. The findings are indicative of appearance of the syndrome of functional myocardial hypodynamia combined with increase in elastic resistance load, as indicated by the absolute extention of EP.

A comparison of periods in the cardiac cycle to nominal values revealed a relative extension of D_o and EP, as well as relative shortening of S_o in the first series.

In the second and third series there was relative extension of S_o and shortening of D_o and EP.

A comparison of the results of loop analysis of the RG revealed that, when breathing oxygen, there was reliable ($P<0.05$) increase in MVRF, PRI, change in shape of RG of the "hypertensive" type (arcuate, absence of extra waves), which is indicative of onset of the syndrome of precapillary hypertension in the pulmonary circulation [8, 11]. Under normoxic hyperbaric conditions there was increase ($P<0.05$) in RDI and PRI, appearance of postcapillary hypertension syndrom characterized by high diastolic waves and M-shaped type of RG. With combined exposure under hyperoxic hyperbaric conditions, we demonstrated the syndrome of mixed hypertension in the pulmonary circulation (PC) with reliable ($P<0.05$) increase in RDI and PRI.

In view of the foregoing, we tried to establish the gradations of pathogenicity of the adverse factors of elevated gas atmosphere pressure, as well as to determine the mechanisms of their effect on MCF.

In the first series, with breathing medical oxygen, the subjects showed a significant extension of the cardiac cycle, mainly due to the diastole, which was apparently attributable to increased tonus of the vagus [16] and change in biochemical processes in the myocardium under the effect of oxygen [17]. On the whole, the dynamics of parameters of functional state of the myocardium in this series of experiments were indicative not only of weakening of contractile function, but decrease in specific energy capacity in the work it performed (extension of D_o, increase in performed work with weakened pumping function of the right ventricle). In our opinion, these changes can be viewed as a manifestation of the principle of economizing cardiac activity under hyperoxic conditions [3].

In the second series, there was appearance of phasic syndrome of myocardial hypodynamia, which is evaluated at the preclinical stages of cardiac insufficiency by most authors as the prime signs of MCF impairment [4] and, in addition, there was increased delivery of blood to the lungs in the diastolic phase [13].

Functional state of the right ventricle with separate and combined exposure to hyperoxia and increased density of gas atmosphere (according to RCG data)

CC phase and estimated param.	Breathing medical oxygen (P = 0.1 MPa)		Breathing nitrogen-oxygen mixture with 4% O_2 (P = 0.5 MPa)		Breathing compressed air (P = 0.5 MPa)	
	background (control)	hyperoxia	background (control)	increased density	background (control)	hyperoxia + increased density
CC, s	1.001±0.005	1.158±0.008*	0.982±0.018	1.118±0.020*	0.968±0.008	1.193±0.011*
AS, s	0.054±0.006	0.076±0.007	0.055±0.002	0.061±0.003	0.049±0.006	0.059±0.004
IC, s	0.032±0.001	0.035±0.002*	0.032±0.001	0.039±0.0009*	0.034±0.001	0.046±0.001*
PS, s	0.085±0.006	0.111±0.005	0.087±0.002	0.100±0.004*	0.083±0.005	0.105±0.004*
RE, s	0.103±0.003	0.114±0.002*	0.103±0.005	0.118±0.006*	0.110±0.002	0.129±0.003*
SE, s	0.204±0.005	0.229±0.006*	0.205±0.008	0.201±0.009*	0.192±0.003	0.195±0.008
EP, s	0.308±0.003 (0.310)	0.344±0.009* (0.334)	0.308±0.005 (0.307)	0.319±0.005* (0.328)	0.302±0.010 (0.304)	0.324±0.008* (0.339)
S_m, s	0.338±0.009	0.378±0.006	0.340±0.004	0.358±0.006	0.336±0.005	0.370±0.009
S_o, s	0.391±0.007	0.454±0.008*	0.395±0.004	0.419±0.005*	0.385±0.014	0.429±0.011*
D_o, s	0.610±0.006	0.704±0.009*	0.587±0.017	0.699±0.014	0.583±0.017	0.764±0.013*
IMC, %	21.9±0.3	24.5±0.3*	22.0±0.8	23.8±0.7	21.8±0.7	24.7±0.9*
ISC, %	90.5±0.5	90.7±0.9*	90.5±0.5	89.1±0.8*	89.6±1.0	87.6±0.9
RSI, %	30.5±0.6	29.6±0.7	31.4±0.3	28.5±0.4	31.2±0.4	27.2±0.6*
MC, units	3.55±0.2	3.09±0.1	3.54±0.03	3.19±0.02*	3.59±0.07	3.06±0.04*
HI, "	0.107±0.004	0.114±0.002*	0.104±0.005	0.118±0.006*	0.113±0.004	0.142±0.007*
EC, "	0.51±0.014	0.49±0.021*	0.51±0.03	0.59±0.028*	0.57±0.01	0.66±0.02*
RDI, %	55.9±2.1	68.7±0.93	57.4±0.53	64.51±0.32*	58.5±0.58	66.8±0.71*
PRI, %	42.3±0.05	45.7±0.06*	46.1±0.04	48.95±0.05*	43.2±0.04	51.5±0.04*
VRF, Ω/s	2.62±0.003	3.02±0.004*	2.70±0.004	2.91±0.006	2.58±0.003	2.74±0.005

Note: Nominal values are given in parentheses: $E_{nom} = 0 \quad 4\sqrt{C} - 0.014$, according to [11].
*P<0.05

Thus, unlike hyperoxia, increased density of the gas environment impaired the phase structure of right ventircular systole, led to decline of MCF and worsening of its functional capacities. The noted changes could be attributed to an increase in inspiratory and expiratory gradients of intrathoracic pressure. This was associated with attenuation of the inhibitory reflex from lung stretching receptors, with appearance of corresponding change in bulboarachnoid mechanisms of regulating cardiac activity and respiration, which is manifested by the "partial vagotonia" effect [2]. Moreover, additional resistance to respiration in the inspiration phase elicits mechanical difficulty of myocardial contraction; in the expiration phase it makes it difficult for diastolic filling of the heart and impairs blood flow in the venous system of the PC. The occurring postcapillary hypertension increases appreciably the load on the right ventricle.

In the third series, with combined exposure to hyperoxia and increased gas density, even more marked signs were demonstrated of diminished contractile function of the myocardium than in the second series. The presence of mixed PC hypertension and maximum changes in recorded parameters in this series warrant the assumption that the MCF changes when breathing compressed air are related to summation of effects of HP factors. The equal severity of EC changes and presence of the functional hypodynamia syndrome in the second and third series, in turn, determine the prevalent effect of density of gas atmosphere over the effect of hyperoxia.

Thus, with brief exposure to HP, the increased density of the gas environment, which causes functional hypodynamia of the myocardium, was the prime factor impairing MCF. Increased density of breathing mixtures elicits postcapillary PC hypertension, while hyperoxia causes precapillary PC hypertension. The factors in question can be arranged in the following order, according to degree of increase in severity of adverse effects on contractile function of the right ventricle: hyperoxia, compressed normoxic nitrogen-oxygen mixture and compressed air.

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CHANGE IN ENERGETICS OF MUSCULAR CONTRACTION AS A RESULT OF HYPEROXIA

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 18, No 4, Jul-Aug 84 (manuscript received 30 Mar 83) pp 77-81

[Article by L. D. Pchelenko and N. A. Bebyakova]

[English abstract from source] Using a highly sensitive thermometric method (1×10^{-5}°C/mm), it was found that the heat production of a single isometric contraction of an isolated diaphragm of the rats exposed to 99% O_2 at normal pressure for 3 and 6 hours significantly differed from the norm: after 3 h hyperoxygenation muscle heat production increased almost three-fold, and after 6 h hyperoxygenation it decreased almost two-fold. The increase is regarded as a result of oxygen-potentiated increase of energy expenditures involved in a contraction and of a decrease of the performance of muscle contraction. The decrease is considered as a consequence of the primary ineconomical energy expenditures by an intensively working muscle represented by the diaphragm and of a reduced viability of the muscle preparation incubated in vitro.

[Text] It is known that oxygen in a specific dose is toxic to all organisms without exception: in protozoans, cell division stops in an atmosphere of pure oxygen; ascarids perish within 1 h of exposure to pure oxygen; when white rats are exposed to a hyperoxic environment for 3 days at normal pressure there is 50% mortality [8]; elevation of partial oxygen pressure in inhaled air to 0.3-0.4 kgf/cm^2 leads to development of oxygen pneumonia in man after 3-6-h exposure [3]. The opinion has been voiced that any increase, even insignificant, in oxygen content of breathing atmosphere, as compared to its level in air, is not indifferent to the body [14]. The threat of oxygen poisoning limits the use of oxygen in space research and for therapeutic purposes. From this point of view, it is of theoretical importance and pressing practical relevance to identify the mechanism of toxic effect of hyperoxia and make a reliable determination of the maximum permissible concentrations of oxygen.

The very fact that oxygen is universally toxic apparently indicates that excessive amounts inevitably lead to impairment of some fundamental vital processes common to all biological systems. The information available in the literature concerning hyperoxic impairment of utilization and transformation

of energy evidently indicates that distortion of normal energy metabolism of cells could be one of the chief causes of oxygen intoxication [1, 9, 11, 12, 17]. If this is true, one should expect changes in heat production of organs and tissues as a direct exogenous indicator of the nature of bioenergetic processes in an organism exposed to the toxic effect of oxygen. The information in the literature on this score is extremely sparse; however, even the existing facts confirm such an assumption either directly or indirectly. It is known that impairment of heat regulation and elevation of body temperature to high levels emerge as an extremely important factor of oxygen pathology [2, 5]. Elevation of ambient temperature during exposure to a hyperoxic factor enhances drastically the toxic effect of oxygen [15]. It was found that sensitivity to oxygen is significantly diminished in heterothermal animals during hibernation, which corresponds to drastic decline of oxygen uptake. On the other hand, there is information to the effect that there is reliable increase in sensitivity to animals in white rats acclimated to the cold (1-0°C for 1 month) [9]. A correlation has been established between increased oxygen uptake and increased electrical activity of rat muscles in a hyperoxic atmosphere (1 h in 60% oxygen at 20-22°C) [10]. However, we do not yet have direct data concerning the levels of heat production by different organs and tissues of animals exposed to a hyperoxic environment. It appears likely that studies to define the exact quantitative energetic changes in an organism exposed to hyperoxia could yield the needed information to form a general idea of oxygen intoxication. Our objective here was to make a quantitative assessment of the effect of single exposure to isobaric hyperoxia on heat production of a single muscular contraction.

Methods

Experiments were conducted on white mongrel female rats weighing 170-240 g. We studied the energetics of heat production of a single isometric contraction of the diaphragm of 3 groups of animals during continuous in vitro incubation at 37°C: the first group consists of 9 control animals kept under vivarium conditions; the second and third groups consisted of rats (7 animals in each) exposed once to 3- and 6-h hyperoxygenation in 99% oxygen.

The experimental protocol was as follows: The animal was sacrificed with rausch anesthesia, then the entire diaphragm was rapidly excised on a ring from the ribs. After thorough preparation, the right half of the muscle was placed on a thermopile mounted in a special frame and attached on the periphery with ophthalmological surgical needles. The central point of the muscle was connected, using a steel hook, to a strain gauge, with which we recorded the muscular exertion (tension) developed by the muscle at the moment of contraction at the point of attachment to the hook. The strain gauge was calibrated with a set of weights, and the tension developed by the muscle was expressed in grams. Stimulating silver electrodes were inserted in the muscle at a distance of 20 mm. The frame with the muscle was placed in a special thermometric system, which was described previously [4]. Before starting the temperature readings, the muscle was allowed to adapt for 40 min to the in vitro incubation conditions, for which purpose the frame with the diaphragm was alternately immersed in Krebs-Henseleit solution decanted on the bottom of the muscle chamber and raised above the solution. Air was blown through

the solution. Finally, the frame with muscle was lifted into the air part of the chamber and, after reaching satisfactory stabilization of temperature in the thermometry system, it was submitted to electric stimulation once every 5 min. The stimulus parameters were 9 V and 16 ms. Calculation was made of head production by a muscular contraction according to the area on graph paper circumscribed by the temperature curve, and it was expressed in mil[illegible] alories. Sensitivity of the thermometry system constituted $1 \cdot 10^{-5}$°C/mm, the co[illegible]rection for the Joule effect, which did not exceed 10%, was taken into consideration in the calculations. Since the muscle preparations were chara[illegible]terized by wide individual scatter of recorded parameters--head production and muscular tension--we added an estimation index characterizing the amount of heat generated by 1 g muscle for each gram of tension developed during contraction, referred to as specific heat production by muscle (q), in order to be able to compare these parameters. We analyzed a total of 73 muscle thermograms.

Additional experiments were performed on 48 animals, 27 of which were in the 1st group, 12 in the 2d and 9 in the 3d. In these animals, we measured respiration rate and rectal temperature using a Temp-1 electric thermometer.

Results and Discussion

The data obtained in this study for all muscle preparations and the mean values for all groups of tested animals are listed in Table 1 (control rats) and Table 2 (experimental rats). As can be seen from these data, even 1 session of exposure to an atmosphere with 99% oxygen drastically altered heat production of a single muscular contraction. Figure 1 illustrates thermograms of muscular contractions, which were recorded in different experiments and so selected as to have approximately the same force of contraction (about 20 g). As can be seen, the muscle of the rat exposed to oxygen for 3 h generated considerably more heat upon contracting than the muscle of an animal exposed to hyperoxygenation for 6 h. On the whole, the results of the studies indicated that, while under normal conditions the rat diaphragm produces about 0.12 mcal heat per unit muscle weight per gram of muscular tension developed, after 3 h of inhaling 99% oxygen the figure increased by 196%, i.e., by almost 3 times, reaching an average of 0.36 mcal ($P<0.01$), whereas after 6 h of hyperoxygenation there was a reliable decline to 0.06 mcal ($P<0.001$), constituting only 52% of the control. These relations between values of contractile heat production by muscles of all three examined groups of animals are illustrated in Figure 2. It also illustrates a diagram of mean values of muscular tension developed by the preparations under given experimental conditions. We see that there is some decrease in force of muscular contraction with regard to muscles of the 2d group of animals (by an average of 16.6%, $P>0.05$) and a

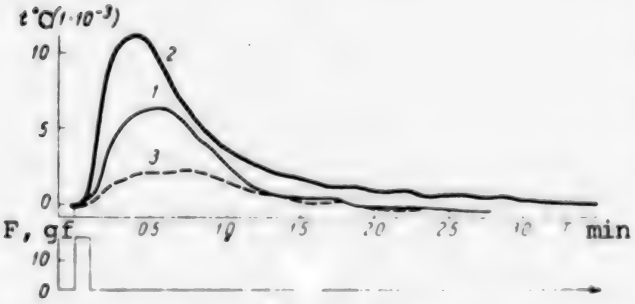

Figure 1. Thermograms of single isometric contraction of isolated rat diaphragm under normal conditions (1) and after 3- and 6-h hyperoxia (2 and 3) recorded in different experiments with equal level of isometric tens[illegible]on (F) developed by muscles

rather significant decline for muscles of animals in the 3d group (by 44%, $P<0.01$). The results of taking rectal temperature failed to reveal significant differences between the animal groups tested, whereas respiration rate increased reliably with both 3-h and 6-h exposure to oxygen (by 27.7 and 25.5%, respectively; $P<0.05$)(Table 3).

Table 1. Heat production, mechanical tension and specific heat production of single isometric contraction of isolated rat diaphragm in control

Preparation No	Muscle weight, g	Rat wt., g	Q, mcal	F, gf	q, mcal/gf•g
1	0,283	240	1,039±0,224	15,8±0,9	0,232±0,007
2	0,406	180	0,833±0,034	40,0±0	0,051±0,002
3	0,320	150	0,367±0,083	15,0±0	0,076±0,007
4	0,252	145	0,240±0,001	16,0±0	0,059±0,006
5	0,324	210	0,413±0,001	12,0±1,0	0,106±0,001
6	0,300	200	0,382±0,058	11,3±0,3	0,113±0,003
7	0,380	225	0,284±0,018	5,0±0,9	0,149±0,002
8	0,354	210	0,998±0,063	12,5±0,5	0,225±0,003
9	0,290	150	0,565±0,077	24,0±1,0	0,081±0,003
$M \pm m$	0,323±0,015	190±13	0,569±0,101	16,8±2,34	0,121±0,021

Key for this and Table 2:
Q) heat production F) mechanical tension q) specific heat production

Figure 2. Mean specific heat production q by muscle and isometric tension F (% of control) during single contraction of isolated rat diaphragm under normal conditions (1) and after 3- and 6-h exposure to atmosphere with 99% oxygen (2 and 3, respectively)

Thus, we found here that the energetics of heat production by a single contraction of the diaphragm of rats under normobaric conditions in an atmosphere of 99% oxygen differed drastically from normal. And, depending on duration of exposure to oxygen in vitro, the effect was reversed: after 3-h hyperoxygenation contractile heat production by the diaphragm almost tripled, with some decline in force of developed mechanical tension, whereas after 6 h of exposure to oxygen, heat production per contraction decreased to one-half, with concurrent significant decrease in force of developed tension. The causes of such drastic changes in energetics of muscular contraction of the diaphragm, a respiratory muscle that functions continuously, within a single session of inhaling oxygen appear to be explainable. Indeed, the muscle fibers of the diaphragm are characterized by high energy metabolism, which is comparable to metabolism of the myocardium [16]. It is known that inhalation of oxygen leads to immediate elevation of its tension in blood and tissues [2, 3, 9]. Polarographic revealed that, while normal oxygen tension in rat muscle constitutes 29±1.0 mm Hg, with brief inhalation of oxygen atmospheric pressure (oxygen test) it rises to 85±2.4 mm Hg within only 4.6 min [7]. The increase in contractile heat production in the

2d group of animals indicates that, in the first hours of exposure to oxygen, the diaphragm spends a total of three times more energy than normally per gram of developed mechanical tension. This indicates significant decline of efficiency of muscular contraction under hyperoxic conditions. However, considering the intensification of respiration rate in rats after 3-h hyperoxia, it can be assumed that the general increase in energy expended per unit time in vivo, i.e., force of heat production during natural function of the diaphragm as a respiratory muscle, increases even more. The demonstrated effect is consistent with the data of V. V. Matsinin [6], who demonstrated intensification of heat flow from the surface of rats exposed to hyperoxibaric conditions [1.2-4.0 atm(abs)] against a background of unchanged rectal and subcutaneous temperatures.

Table 2. Heat production, mechanical tension and specific heat production of single isometric contraction of isolated rat diaphragm after 3- and 6-h hyperoxia

Prep. No	Muscle weight, g	Rat wt., g	Q, mcal	F, gf	q, mcal/gf•g
			3-h hyperoxia		
1	0,331	190	1,466±0,080	19,5±1,3	0,221±0,012
2	0,319	200	3,389±0,260	19,8±1,6	0,537±0,041
3	0,248	180	1,905±0,172	12,5±1,0	0,614±0,055
4	0,455	180	1,065±0,011	13,0±1,3	0,180±0,002
5	0,237	205	0,763±0,010	8,0±1,0	0,402±0,005
6	0,254	190	0,742±0,015	8,0±1,5	0,365±0,007
7	0,442	240	1,240±0,011	15,0±0	0,187±0,002
$M\pm m$	0,327±0,032	206±15	1,511±0,347	13,7±1,8	0,358±0,065
			6-h hyperoxia		
1	0,373	175	0,256±0,010	10,5±0,5	0,065±0,002
2	0,279	170	0,131±0,001	8,5±0	0,055±0,004
3	0,283	160	0,132±0,002	7,0±1,5	0,067±0,001
4	0,270	160	0,120±0,001	10,0±0	0,044±0
5	0,391	210	0,187±0,004	6,0±1,0	0,080±0,002
6	0,385	170	0,176±0,002	9,0±1,0	0,051±0,001
7	0,233	110	0,253±0,010	15,0±2,0	0,076±0,003
$M\pm m$	0,315±0,024	165±12	0,179±0,025	9,4±1,1	0,063±0,005

Table 3. Rectal temperature and respiration rate under normal conditions and after hyperoxia (additional experiments)

Parameter	Rat group		
	1	2	
Respiration rate/min	130 ± 8,3	[illegible]	104 ± 13,2
Rectal temperature, °C	38,2 ± 0,3	38,8 ± 0,3	38,4 ± 0,6

Further exposure of white rats to oxygen led to marked decline in contractile head production and force of muscular contraction of the diaphragm. It can be assumed that these changes were the consequence of initial uneconomical expenditure of energy during the first hours of exposure, enhanced by oxygen, and absence of appropriate compensatory reactions during in vitro intermittent incubation, which are implemented by blood flow in vivo. In our opinion, this assumption is consistent with data in the literature to the effect that there is drastic depletion of macroergs and impairment of oxidative phosphorylation processes during hyperoxia [11, 12, 17]. It was established that, during the period of heightened functional activity of cells, there is some discoordination of respiration and phosphorylation in a hyperoxic environment, in the direction of more intensive respiration [1]. According to the hypothesis of Fisher et al. [13], the mechanism of action of oxygen is such that there is primary damage to oxidation substrates involved in reactions related to production of energy. The ultimate cause of cell death under hyperoxic conditions is the catastrophic decline of ATP.

On the whole, our experiments showed that the energetics of heat production by an intensively functioning muscle are extremely sensitive to even one exposure to normobaric hyperoxygenation, changing in the direction of increased expenditure of energy and, consequently, increased energy cost of a muscular contraction.

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HEMODYNAMICS AND NEURODYNAMICS OF THE HUMAN BRAIN DURING EXPOSURE TO MODERATE HYPOXIC HYPOXIA

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 18, No 4, Jul-Aug 84 (manuscript received 1 Feb 83) pp 81-86

[Article by D. A. Alekseyev, A. F. Zubarev, T. N. Krupina, Kh. Kh. Yarullin, Ye. I. Kuznets and M. P. Kuz'min]

[English abstract from source] Synchronous electro- and rheoencephalography were used to study tolerance to moderate hypoxic hypoxia for 30 min at an altitude of 5000 m without additional oxygen supply. As test subjects, men with autonomic-vascular dystonia (29-39 years old), 15 men over 40 (41-56 years old) and 16 essentially healthy controls (23-36) were used. The aged volunteers (41-56 years old) did not differ from the controls with respect to their tolerance to hypoxic hypoxia. The men with early symptoms of hypertonic-type dystonia also showed high tolerance to hypoxic hypoxia. The subjects with hypotonic-type dystonia displayed lower tolerance.

[Text] The test with "ascent" to an "altitude" of 5000 m in a pressure chamber is a mandatory expert screening test in aerospace medicine [4], since this makes it possible to assess tolerance of hypoxia, particularly cerebral. At the same time, heretofore this expert test included evaluation of only parameters (appearance, behavior, respiration rate, pulse rate, arterial pressure, ECG) that permit evaluation of functional state, but are not very informative with regard to assessing man's reserve capabilities. Assessment of physiological reserve capacities and adaptive reactions to hypoxic hypoxia is particularly important for older individuals with partial deficiency of health status [10].

Methods

A study was made of regional hemodynamics of the brain (rheoencephalographic method) and its bioelectrical activity (electroencephalographic method) in 16 essentially healthy young men 23-26 years of age (control group), 10 subjects with early manifestations of vegetovascular dystonia (VVD) 29-39 years of age and 15 men over 40 years old (41-56 years) during exposure to an "altitude" of 5000 m in a pressure chamber in a relatively quiet state for 30 min without additional supply of oxygen. The methods for recording and analyzing

rheoencephalograms (REG) and electroencephalograms (EEG) have been described before [1, 2]. Concurrently with the rheoencephalographic and electroencephalographic studies, in addition to conventional physiological monitoring methods (respiration rate, pulse rate, arterial pressure, ECG), we determined acid-base equilibrium (pH) and gas composition of blood (pO_2 and pCO_2) by a micro-express method using an Astrup apparatus (Radiometer Firm, Denmark). Blood was drawn from the tip of a finger that had been warmed.

Results and Discussion

Similar changes in acid-base equilibrium and gas composition of blood developed in all of the subjects (Table 1) during the hypoxia test. By the 30th min of exposure to moderate hypoxic hypoxia, there was a shift in the active reaction of blood in the direction of respiratory alkalosis (blood pH increased by an average of 0.029; $P<0.05$). Blood oxygen tension dropped by a mean of 43.8 mm Hg (by 46.8%; $P<0.05$), i.e., consistent with the magnitude and duration of hypoxia, whereas carbon dioxide tension dropped by only 3.9 mm Hg (9%), which was indicative of some hyperventilation by the subjects.

Table 1. Dynamics of pH and blood gas (pO_2 and pCO_2) parameters while breathing in an atmosphere with low partial oxygen pressure (mean values; n = 41)

Parameter	Background	Diminished hypox. hypoxia (30 min)	Recovery period (30th min)
pH	7,400 ± 0,003	7,429 ± 0,009	7,395 ± 0,004
pO_2, mm Hg	82,6 ± 1,0	35,6 ± 1,3	39,6 ± 1,0
pCO_2, mm Hg	39,5 ± 0,4	35,6 ± 1,3	39,6 ± 1,0

According to the classification of hypoxic hypoxia tolerance [4], it was good in 11 subjects, satisfactory in 26 and low in 4.

The subjects with good tolerance presented no complaints during the test, their wellbeing remained normal and their appearance did not change. Their heart rate increased by 10-13/min under hypoxic conditions.

The most typical complaints of individuals with satisfactory tolerance of hypoxia were general weakness, mild vertigo, drowsiness, sensation of blood rushing to the neat and pulsation in the temples. Objectively, there was moderate pallor of the integument and mild cyanosis of the lips. Their heart rate increased by an average of 14/min. In most cases, the subjects' well-being and appearance improved during the second half of the test.

During the test, subjects with good and satisfactory tolerance (mainly healthy individuals in all age groups) presented noticeable compensatory dilatation of cerebral vessels (dicrotic and diastolic REG indexes decreased by 20-30%; $P<0.05$), which was associated with adequate increase in pulsed delivery of blood to the brain (Table 2), both in the bed of the internal carotid and vertebrobasilar system (maximum REG amplitude in the frontomastoid lead increased by an average of 60%; $P<0.05$, and in the bimastoid lead by 26% of base value).

Table 2. Dynamics of REG parameters in frontomastoid (F-M) and bimastoid (M-M) leads in subjects with satisfactory tolerance of hypoxia (n = 26)

Parameter	Background	Hypoxic hypoxia, min							Recov. period (10th min)
		1	5	10	15	20	25	30	
A, (Ω) F-M	0,091± 0,009	0,118± 0,008	0,109± 0,009	0,127± 0,007	0,145± 0,011	0,127± 0,007	0,123± 0,012	0,113± 0,012	0,107± 0,012
A, (Ω) M-M	0,113± 0,011	0,113± 0,01	0,118± 0,008	0,128± 0,008	0,142± 0,012	0,141± 0,005	0,136± 0,006	0,135± 0,007	0,125± 0,013
DCI, % F-M	56,8± 2,5	54,1± 2,7	50,7± 2,1	46,6± 1,8	44,3± 1,6	39,6± 1,9	43,6± 2,4	49,2± 2,0	61,2± 2,3
DCI, % M-M	50,9± 1,8	49,1± 2,3	46,1± 1,7	42,0± 3,7	38,4± 1,2	45,4± 1,1	46,8± 1,5	49,3± 1,6	56,0± 2,3
DSI, % F-M	64,6± 2,0	65,1± 2,3	58,9± 1,8	56,1± 2,4	53,1± 2,5	51,4± 2,1	54,2± 1,8	57,6± 1,6	66,9± 1,9
DSI, % M-M	60,8± 1,7	59,3± 2,1	57,2± 1,6	53,5± 1,5	49,1± 1,3	53,0± 1,9	54,7± 1,7	55,8± 2,0	66,9± 1,8

Key: A) maximum REG amplitude (in Ω) DSI) diastolic index (%)
DCI) dicrotic index (%)

In this group of subjects there were minimal changes in bioelectrical activity of the brain, and in most cases they were limited to brief, moderate desynchronization followed by synchronization of α rhythm. However, there was no reliable increase in amplitude and index of dominant activity, as compared to background values, in the synchronization phase.

Subjects with early manifestations of VVD of the hypertensive type presented satisfactory tolerance of hypoxic hypoxia. Although there was a distinct decrease in tonus of cerebral vessels, which was the most marked in the 10th-15th min of the test, pulsed delivery of blood to cerebral vessels did not change appreciably. Concurrently, we observed more marked changes in bioelectrical activity of the brain than in individuals with good tolerance of the test (Figure 1). Sporadic appearance of sharp-wave, spike or paroxysmal activity during hypoxia was observed in 3 subjects with VVD of the hypertensive type and 2 who were over 40 years of age.

The subjects with diminished hypoxia tolerance complained of vertigo, sensation of unreality of what was happening, febrile feeling all over the body and increased perspiration. Objectively, there was marked pallor of the integument and cyanosis of the lips. Heart rate increased by 23-25/min. These signs developed in the first 5-10 min of exposure to hypoxia, with subsequent improvement of wellbeing and general condition.

In subjects with low tolerance of the test and with VVD of the hypotensive type, there was progressive decrease in tonus of cerebral vessels with increase in stay in the presssure chamber. The increase in pulsed filling of cerebral vessels was relatively brief and, starting in the 2d-3d min of hypoxia, it and particularly its mean fluctuations (according to area of REG wave) diminished, apparently because of accentuation of systemic hypotensive reaction and relative insufficiency of minute blood volume. Indeed, in this group of subjects, judging by the dynamics of finger rheograms, there

was marked decrease of arteriolar and venous tonus in peripheral vessels, as well as (in some subjects) decline of wave voltage on the ECG.

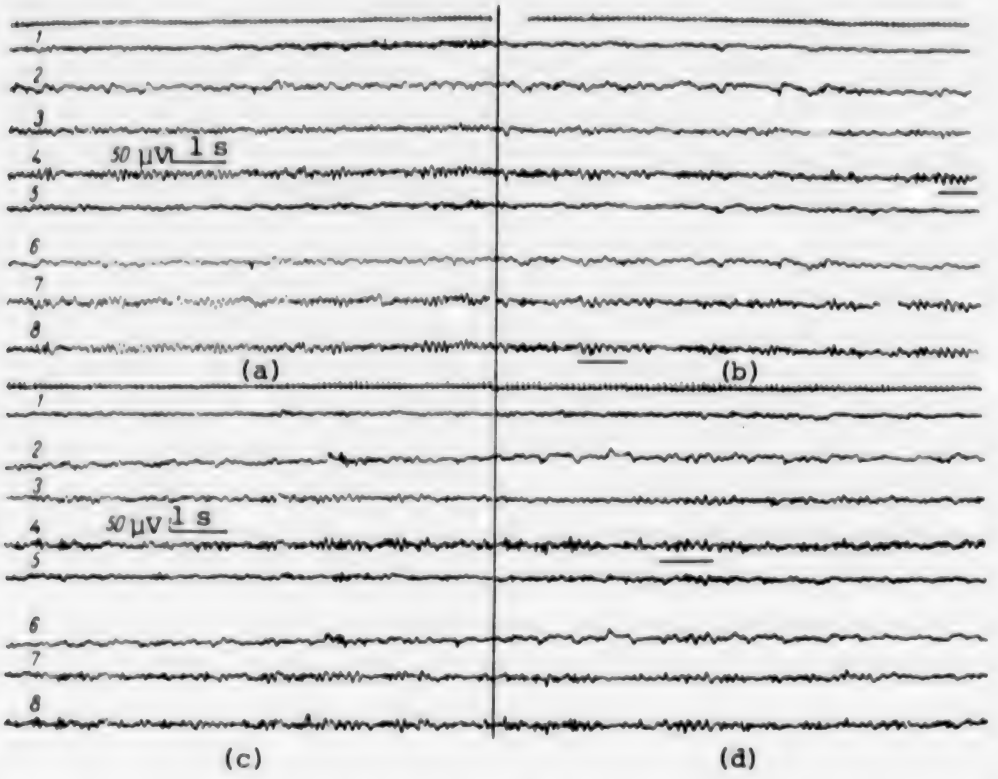

Figure 1. Bioelectrical activity of the brain of subject G-v, 39 years old, with early manifestations of VVD of the hypertensive type

a) before hypoxia test
b, c, d) 10th, 20th and 30th min of test
Here and in Figure 2, top to bottom:
1, 2) left and right frontoparietal EEG leads
3, 4) occipitoparietal
5, 6) frontotemporal
7, 8) occipitotemporal

Slow bioelectrical activity is underscored.

In this group of subjects, the EEG was characterized by significant irritation of bioelectrical activity, which was followed in some cases by a phase of desynchronization of bioelectric potentials in the β rhythm with appearance of group "bursts" of β waves (14-20 cps, to 70 μV) and polymorphic slow, high-amplitude activity. While none of the subjects in the control group presented pathological forms of activity during the test, those with diminished tolerance for hypoxic hypoxia showed periodic appearance of sharp-wave, spike or paroxysmal slow activity in half the cases (Figure 2). Their slow wave activity increased on the average from 7.8±2.8 to 53.6±6.9 μV ($P<0.05$). In the other subjects it did not exceed 35 μV.

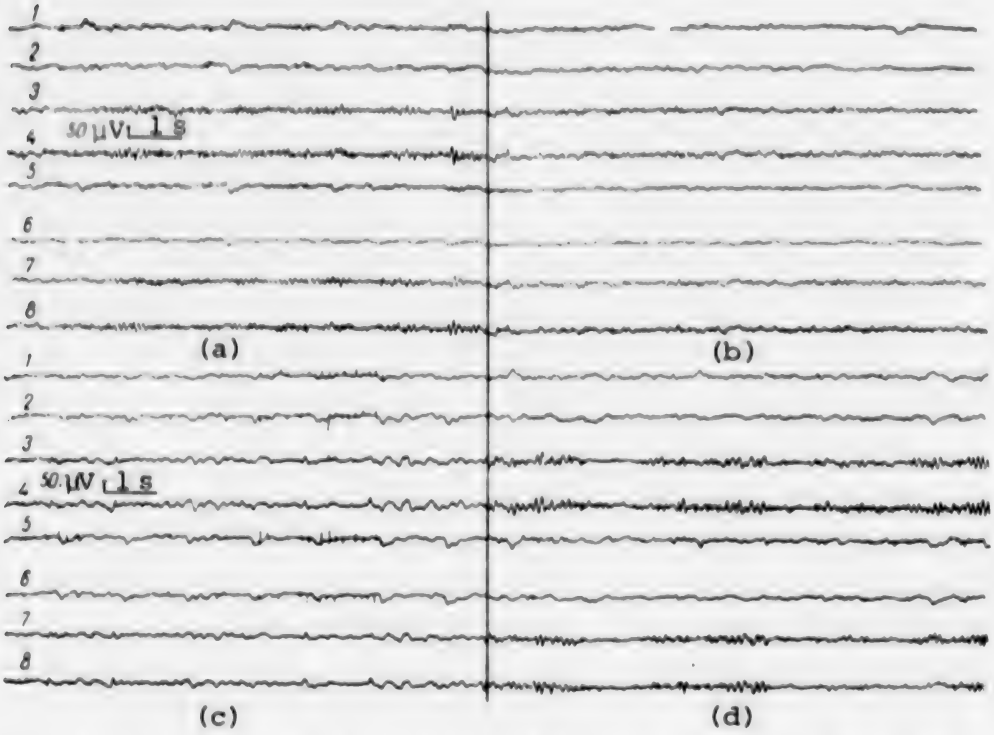

Figure 2. Bioelectrical activity of brain in subject M-v, 30 years old, with early manifestations of VVD of hypotensive type

a) before hypoxia test
b,c) 5th and 15th min of test
d) 1st min of recovery period

Other designations are the same as in Figure 1.

Thus, there was rather high tolerance of hypoxic hypoxia in individuals with signs of VVD of the hypertenisve type; in those with VVD of the hypotensive type tolerance for the test was low, while healthy subjects over 40 years of age showed virtually no difference from the control group with regard to hypoxia tolerance.

V. I. Plakhatnyuk [12] has shown that individuals suffering from VVD of the hypertensive type tolerate well the hypoxia test, unlike those, in whom regulatory disturbances of the cardiovascular system are of the hypotensive type.

On the whole, the changes in pulsed delivery of blood, tonus of cerebral vessels and bioelectrical activity of the brain were phasic. The compensation phase corresponded to increased pulsed delivery of blood to the brain and some desynchronization of its bioelectrical activity. With worsening of compensation, there were marked changes in cerebral hemodynamics and neurodynamics: rapid decrease in tonus of cerebral vessels, diminished pulsed delivery of blood, increase in polymorphic slow activity on the EEG against a background of more marked desynchronization or appearance of marked synchronization of bioelectric potentials and occasional demonstration of pathological forms of activity.

Analysis of individual distinctions of background EEG and REG revealed that, in individuals with minor deviations from the base state of pulsed delivery of blood and tonus of cerebral vessels, as well as bioelectrical activity of the brain, there were more marked changes during the test than in those whose background EEG and REG were in the normal range (see Figure 1). In other words, we established a correlation between base tonus and pulsed filling of cerebral vessels, bioelectrical activity of the brain and tolerance of the test. This enabled us to consider the question of predicting hypoxic tolerance of subjects with some distinctions in their base state of cerebral hemodynamics and neurodynamics. The results of our study enabled us to demonstrate rheoencephalographic and electroencephalographic parameters of tolerance of the test with "ascent" in a pressure chamber to an "altitude" of 5000 m (with exposure for 30 min) at relative rest, without additional oxygen supply.

The following are indicators of heightened sensitivity of the brain to hypoxic hypoxia (diminished tolerance of the test): 1) according to REG data--progressive decrease in cerebrovascular tonus associated with diminished pulsed delivery of blood to the brain; 2) according to EEG data--marked decrease in amplitude (more than 40% in relation to base value), frequency (more than 25% in relation to base value) and index (more than 65% in relation to base value) of α activity; smoothing (more than 70% in relation to base data) or inversion of zonal differences; appearance of synchronization of bioelectric potentials of the brain for β rhythm; progressive increase in diffuse slow activity (with amplitude over 50 μV) or appearance of bilaterally synchronous paroxysmal slow activity; appearance of sharp waves and spikes.

The results of synchronous REG and EEG studies demonstrated relative stability of hemodynamics and neurodynamics of the brain in healthy subjects exposed to moderate hypoxic hypoxia. At the same time, REG and EEG dynamics in subjects with early manifestations of VVD of the hypertensive and, particularly, hypotensive type were indicative of weakening of mechanisms of regulating cerebral circulation and increased sensitivity of brain structures to moderate hypoxic hypoxia. In other words, there were changes in metabolic and neurogenic mechanisms of myogenic regulation of cerebral circulation in subjects with early manifestations of VVD, unlike healthy subjects, with exposure to moderate hypoxic hypoxia. This is consistent with the data of Kogure et al. [16] and Hamer et al. [13], who demonstrated that a change in oxygen concentration in inspired air also changes the mechanisms of regulating cerebrovascular tonus.

It is a known fact that changes in pO_2 and pCO_2 in brain structures are important to processes of autoregulation of cerebral circulation. When pO_2 drops (under normocapnic conditions) there is intensification of delivery of blood to corresponding brain structures, whereas with elevation of pO_2 there is decrease in cerebral blood flow; a decline of pCO_2, unlike the dynamics of pO_2, elicits a decrease in cerebral blood flow, while hypercapnia increases it [5, 7, 16, 17]. Since the decline of arterial blood pO_2 was considerably more marked in our study than the decline of pCO_2 (see Table 1), it can be considered that there was compensatory vasodilatation and adequate increase in pulsed delivery of blood to the brain. This is consistent with data to the effect that there is redistribution of blood with increase in delivery of blood to vital organs, i.e., brain and heart, under hypoxic conditions [5, 9, 11, 14]. The mechanisms of such cerebral vasodilatation under

hypoxic conditions are described by Kogure et al. [16]. The more significant increase in delivery of blood to the cerebral hemispheres, as compared to the vertebrobasilar system, is apparently attributable to the greater sensitivity to hypoxia of the phylogenetically younger brain structures [3, 6, 8, 15].

The demonstrated EEG and REG parameters of the subjects' tolerance of moderate hypoxic hypoxia, particularly signs of cerebral hypoxia and hypovolemia, may be of diagnostic and prognostic importance in settling questions of expertise for evaluation of tolerance of pressure chamber tests in professional screening.

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TOXICOLOGICAL EVALUATION OF COLUMBIA SPACECRAFT

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 18, No 4, Jul-Aug 84 (manuscript received 4 Aug 83) pp 87-96

[Article by W. J. Rippstein and M. E. Coleman (United States)]

[English abstract from source] Atmospheric contamination of spacecraft crew cabins has been a toxicological concern since this country began its efforts in manned space flight [1]. Procedures have been developed and utilized for determining the identities and quantities of contaminant gases present in the crew cabin environment. Methods have also been developed for assessing and controlling the trace gas contaminant buildup within the closed environment of spacecraft cabins. Although nearly one hundred contaminant gases have been detected in the Shuttle crew cabin, for the most part, the concentrations of these gases have been maintained below a toxicity hazard level.

[Text] Toxicological support of the space program of missions aboard a reusable transport spacecraft (MTKK) [shuttle] was essentially the result of experience acquired in the course of prior NASA space programs. Experience was gained mainly in the course of the manned spaceflights on the Apollo Program. At that time, it was believed that nonmetallic materials should be used to build the Apollo spacecraft. The program for construction materials selection involved not only investigation of materials that were promising from the standpoint of flammability, but assessment of their toxicity hazard, which could be present due to outgassing of contaminants from the construction materials into the crew cabin environment. Analysis of gases discharged from the nonmetallic materials of Apollo made it possible to detect and identify more than 300 different compounds. At that time, it was established that the types and amounts of contaminants outgassed from materials in the spacecraft cabin should be strictly controlled to assure reliable protection of crews against a potential toxicity hazard.

The same program for materials selection was adopted at the early stages of development and manufacture of the MTKK orbital stages. Outgassing analyses were made on materials such as fluids in the heat exchanger and fire extinguisher, thermal insulation, electrical wire insulation, paint, lubricants, adhesives, plastics, various types of rubber and elastomers. In addition, much attention was given to evaluation of the selected nonmetallic materials with respect to the composition of combustion products. The combustion products

of these materials were of particular interest because of the highly toxic gases usually produced during combustion. The choice of most of the nonmetallic materials for construction of the orbiter was based on their ability to resist combustion at high ambient temperatures. These criteria usually led to selection of materials consisting of halogenated and nitrogenated hydrocarbons, which increase the toxicity of a material upon combustion in most cases.

Two other areas of toxicology of importance to SS missions pertained to skin damage from contact with toxic substances and digestive system disturbances due to ingestion of toxic substances. No doubt, the most difficult toxicological problems that had been encountered to date were problems of inhalation toxicology--inhalation of outgassed contaminants and products of combustion of nonmetallic construction materials. For this reason, questions of toxicology on the Space Shuttle Program that are related to damage to the digestive system (due to penetration of toxic agents into the body) and the skin (due to contact with toxic agents) will be covered in a separate work.

Methods

The toxicological program that will be followed in missions of the Space Shuttle MTKK consisted of five major problems:

1. Determination of toxicity standards for the atmosphere during spaceflights.

2. Elaboration of a program for selection of construction materials.

3. Development of methods for removing contaminants from the MTKK environment.

4. Development of methods and performance of measurements of levels of contaminants in the MTKK atmosphere.

5. Development of principles and methods for toxicological testing of the habitable environment aboard MTKK.

Establishment of Spaceflight Atmosphere Toxicity Standards

In order to determine the maximum permissible concentrations of contaminant gases or gas mixtures in the atmosphere of MTKK cabins, it was necessary to develop new standards. Since crew safety and success of a mission depend largely on work capacity of cosmonauts, criteria for behavioral reactions, rather than classical, time-averaged, weighted mean or threshold values for permissible concentrations of toxic agents, usually served as the basis for developing toxicity standards for the MTKK cabin atmosphere [2].

For this reason, new data were needed about the toxic substances contained in inhaled air, as related to spaceflight conditions, since most of the existing information in the area of inhalation toxicology pertains to 40-h per week exposure. Since the crews of both spacecraft and submarines work in a closed environment for long periods of time, analogous problems often arise in both situations, which are related to the atmosphere of closed environments. For this reason, maximum permissible concentrations of toxic compounds are often the same for spacecraft and submarine environments.

In view of the lack of sufficient information needed to investigate the short-term (on the order of a few days) continuous effects of trace concentrations of many toxic compounds contained in the atmosphere, representatives of the National Academy of Sciences asked for the assistance of toxicologists from different organizations. A list of the known trace contaminant gases present in the MTKK atmosphere were submitted for examination by a special committee of the National Academy of Sciences. The committee consisted of toxicologists from government institutions, scientific research institutes and industrial organizations. The values they recommended constituted, in most cases, one-half to one-tenth the concentrations adopted as the standard of maximum permissible concentrations of toxic compounds in the atmosphere of premises, in which people worked for 40 h per week. These levels were adopted as the maximum permissible concentrations of toxic substances in the MTKK atmosphere.

Development of Programs for Selection of Construction Materials

The second phase of work on toxicology included development of a program for controlling the selection of materials used aboard the MTKK on the basis of outgassing characteristics of these materials. Several criteria were established to determine methods of creating conditions for testing materials selected as candidates for use aboard the MTKK. The most important information gained in the course of the tests consisted of parameters for determining the quantitative and qualitative composition of outgassed compounds from each material. Then the materials were analyzed for rate of outgassing of each identified compound. The criteria, according to which a construction material proposed for use aboard a spacecraft was accepted or rejected, were outgassing characteristics, size of MTKK cabin, duration of flight, maximum allowable concentrations, capabilities of the system for controlling atmosphere parameters (ARS) to remove trace amounts of toxic substances from the MTKK atmosphere.

Development of Methods for Removal of Contaminants From MTKK Atmosphere

The purpose of the third part of the toxicological program was to assure removal of contaminant gases by means of a system for controlling MTKK atmosphere parameters. As a result of the joint efforts of specialists, three methods were developed to remove contaminant gases by means of the MTKK system for controlling atmosphere parameters.

The first method is based on gas absorption by an absorbent filter made of activated carbon contained in a column with lithium hydroxide to remove carbon dioxide. This column is also used to remove some gases containing hydrogen sulfide from the cabin atmosphere. The second method involves the use of a specially designed device known under the name of catalytic oxidizer in the system for controlling ambient temperature. The catalytic oxidizer consists of two absorbent filters placed one after the other, made of activated charcoal, with platinum coating on the second filter. The platinum-coated carbon acts as a catalyst for the ambient temperature control system, transforming carbon monoxide contained in the cabin atmosphere into carbon dioxide. Carbon dioxide is absorbed from the airstream by means of the column with lithium hydroxide. Some trace amounts of gases are removed by the absorbent activated carbon filter, the catalytic oxidizer of the ambient temperature control system. To

remove trace contaminant gases from the spacecraft atmosphere by the third method, use is made of the gas dehumidifier of the onboard system for control of parameters of spacecraft atmosphere. Relative air humidity in the cabin is controlled by passing air over a cooled surface. In this way, the water is condensed on this surface and removed.

Trace amounts of water-soluble contaminant gases are removed together with water from the gas dehumidifier.

Development of Methods and Measurement of Levels of Contaminants in MTKK Atmosphere

The fourth stage of the toxicological program involves development of methods and procedures for analytical measurement of levels of trace contaminants in the spacecraft atmosphere. On the basis of previously gained experience in evaluating the composition of the atmosphere of closed environments in the course of tests in a pressure chamber and prior analyses of spacecraft cabin atmosphere, it was concluded that two methods would be used to obtain full quantitative and qualitative information about the atmosphere of a spacecraft. These methods are known under the names of whole-gas (first method) and absorbed-gas (second method) tests. Both are used to evaluate the atmosphere in the crew cabin atmosphere of MTKK both on the ground and during missions. The first method is used for instant collection of air samples, whereas the second is used to collect air samples continuously.

The first method of collecting samples on the ground requires use of a pressure pump to transfer air samples into a stainless steel tank. When it is necessary to take a sample, the valve on the tank is opened for an instant and air is drawn into the tank.

The procedure for collecting samples under ground-based conditions using the second method requires pumping air samples taken in the cabin through tanks containing a substance known under the name of Tenax. This material can adsorb most contaminant gases from the air and it has the unique capacity of allowing air vapor to pass through it.

In flight, space vacuum is used to pass cabin air through the substrate (Tenax) contained in the tank, which has excellent absorption properties.

The air samples taken by both methods on the ground and in flight are forwarded to a laboratory for analysis. Chemical analyses of air samples are made by gas chromatography and a combined method, which includes gas chromatography and mass spectrometry. Quantitative assays are made mainly by standard methods: gas chromatography combined with mass spectrometry, whereas qualitative evaluations are made by standard gas chromatography techniques. Due to the concentrating effect of the second method, it is used mainly for qualitative analyses. The first method is the most accurate, since the samples in the tank contain the very same concentrations of trace contaminants as those present in the atmosphere at the time the samples were taken.

Elaboration of Guidelines and Methods for Conducting Toxicological Studies of MTKK Crew Environment

When conducting most toxicological studies, which include assays of trace gas contaminants, either only one or (sometimes) several gases are considered at one time. When evaluating the atmosphere of a spacecraft cabin with regard to trace toxic gases, it was assumed that there could be about 100 trace contaminant gases present in the craft environment during a flight. The plan was that Shuttle missions would last no more than 7 days. The levels of maximum allowable concentrations established for spaceflight conditions were determined on the basis of the following criteria:

1) Continuous exposure for a total of up to 7 days; 2) exposure to a single contaminant gas; 3) absence of other stress factors, for example, heat, cold, disease, trauma, etc.; 4) in the absence of data about the toxicity of a compound under study, maximum allowable concentration for a spacecraft is determined for the compound on a level that equals the toxicity of the most toxic compound in the group.

In order to make a toxicological assessment of the results obtained on the basis of analysis of trace contaminant gases outgassed into the MTKK cabin atmosphere, the trace gases were distributed into groups, according to the nature of their toxicological effect on man. The following groups of trace contaminants were identified: compounds that have an irritant effect, for example, aldehydes and ammonia; asphyxiants, for example, carbon monoxide and methane; depressants of central nervous system activity (anesthetics and narcotics), for example, ethers, ketones, alcohols and halogenated hydrocarbons; systemic poisons, for example, benzenes, phenols and naphthalanes; particles of solids, for example, silicon and asbestos.

The compounds listed above can shift from one group to another, depending on concentration. Moreover, they may demonstrate physiological effects that are inherent to compounds referable to several groups. In addition, the physiological effects of compounds within a given group could be additive, synergistic or subtractive. There is no information about the synergistic or subtractive effects of about 100 gases detected in the MTKK cabin. For this reason, in order to make an overall evaluation of the MTKK cabin atmosphere, we considered only the additive effects of a given physiological group. Since the MTKK atmosphere parameter control system has a particulate filter, the solid contaminants in the atmosphere were not recorded inflight. For this reason, when assessing the toxicity of contaminants in the crew cabin atmosphere, assays of such compounds were not made.

Each of the four categories of physiological effects was evaluated on the principle of group limit. For this purpose, determination was made of the sum of ratios of actual concentrations recorded in the cabin to concentrations corresponding to maximum allowable concentrations of trace contaminant gases in the cabin atmosphere. The sum obtained for each group of compounds studied should not exceed 1 in the case where a safe environment has been provided in the cabin. The following mathematical expression is used to describe these conditions:

$$0 < \frac{C_1}{SMAC_1} + \frac{C_2}{SMAC_2} + \frac{C_3}{SMAC_3} + \ldots + \frac{C_n}{SMAC_n} < 1 \text{ or}$$

$$0 < \sum_{i=1}^{n} \frac{C_i}{(SMAC)_i} < 1,$$

where C is concentration of contaminant gas and SMAC (spacecraft maximum allowable concentration) is maximum permissible concentration of a compound for a spacecraft.

Results and Discussion

On the basis of samples taken during the first 5 MTKK missions, 157 different trace contaminant gases were identified and assayed. The first 5 missions were performed using the same MTKK (Columbia). Table 1 lists the identified compounds detected in the Shuttle atmosphere and the missions during which they were detected. Tables 2-6 list data referable to qualitative and quantitative analyses of air samples collected during the first 5 MTKK flights.

Table 1. Contaminants found in air samples taken in MTKK cabin

Compound	MTKK mission				
	1	2	3	4	5
Acetic acid, n-butyl ester	+				
Acetic acid, 2-ethoxyethylester	+				
Benzaldehyde		+			
Benzene	+	+	+		+
Bromotrifluoromethane			+	+	
1-Butanal	+	+	+		
1-Butanol	+	+			
2-Butanone	+	+	+		+
Butene					+
n-Butylbenzene	+				
Carbon disulfide		+			
Carbon monoxide	+	+	+		+
Cyclohexane		+			
Decane		+			
Dichlorodifluoromethane		+		+	
1,1-Dichloroethane		+			
Dichloromethane	+	+	+		+
1,2-Dimethylbenzene	+	+	+		
1,3-Dimethylbenzene	+	+	+		+
1,4-Dimethylbenzene	+				+
1,1-Diethylethanol	+				
Ethanal	+	+	+		+
Ethanol	+	+	+	+	+
Ethylbenzene	+	+	+		
2-Ethylhexanal		+			
1-Heptanal	+				
Heptane		+	+		

Table 1. (continued)

Compound	MTKK mission				
	1	2	3	4	5
2-Heptanone	+				
3-Heptanone	+				
Hexamethylcyclopentane		+			
Hexamethylcyclotrisiloxane	+				
1-Hexanal	+				
Hexane	+	+			
Indan		+			
Methane	+	+	+	+	+
Methanol	+	+			
2-Methyl-1,3-butadiene	+				
Methylcyclopentane	+	+			
Methylethylcyclopentane			+		
6-Methyl-2-heptanone		+			
2-Methylpentane		+			
2-Methyl-1-propanol		+			
2-Methyl-2-propanol		+	+		
4-Methyl-2-propantanone	+		+		
Naphthalene		+			
Nonane		+			
Octane		+			
1-Pentanal	+	+	+		
Pentane		+	+		
1-Propanal	+	+	+		
2-Propanol	+		+		
2-Propanone	+	+	+	+	+
Prophylbenzene	+	+			+
Toluene	+	+	+		+
1,1,1-Trichloroethane	+	+	+		
Trichloroethane		+	+		
Trichlorofluoromethane	+	+	+		
1,1,2-Trichloro-1,2,2,-trifluoroethane	+	+	+	+	+
Trimethyl silanol		+			
C_7-aliphatic hydrocarbons (1)		+			
C_8-aliphatic hydrocarbons (7)		+			
C_9-aliphatic hydrocarbons (9)		+			
C_{10}-aliphatic hydrocarbons (8)		+			
C_{11}-aliphatic hydrocarbons (8)		+			
C_{12}-aliphatic hydrocarbons (8)		+			
C_{13}-aliphatic hydrocarbons (1)		+			
C_{14}-aliphatic hydrocarbons (13)		+			
C_8-alkane (1)				+	
C_9-alkane (4)				+	+
C_{10}-alkane (6)	+		+		
C_{11}-alkane (5)	+		+		
C_{12}-alkane (4)	+		+		
C_8-olefinic hydrocarbon (1)		+			
C_9-olefinic hydrocarbon (2)		+			
Siloxane (3)					
C_3-substituted benzene (11)	+				
C_4-substituted benzene (6)	+				

Note: Number of compounds identified in each given category is given in parentheses.

Table 2. Analysis of atmosphere in MTKK-1 cabin

Compound	Cylinder			
	1	2	3	4
Carbon monoxide	<0.572 (<0,5)	<0.572 (<0,5)	<0.572 (<0.5)	1.019 (0.890)
Methane	4.645 (7.10)	8.309 (12.70)	1,439 (2.20)	18.384 (28.10)
Trichlorofluoromethane	—	—	0.021 (0.004)	—
1,1,2-Trichloro-1,2,2-trifluoroethane	0.943 (0.124)	0.129 (0.017)	2.312 (0.304)	5.697 (0.749)
Ethanal	0.116 (0.064)	0.104 (0.058)	0.069 (0.038)	0.142 (0.079)
2-methyl-1,3-butadiene	0.032 (0.012)	0.022 (0.008)	0.005 (0.002)	0.028 (0.010)
n-Hexane	—	—	0.039 (0.011)	—
Methylcyclopentane	—	—	0.150 (0.044)	0.041 (0.012)
Propanal	0.042 (0.018)	0.043 (0.018)	0.041 (0.017)	0.076 (0.032)
2-Propanone	0.096 (0.041)	0.092 (0.039)	0.066 (0.028)	0.166 (0.070)
n-Butanal	0.029 (0.010)	0.035 (0.012)	0.027 (0.009)	0.085 (0.029)
2-Butanone	0.024 (0.008)	0.018 (0.006)	0.074 (0.025)	0.044 (0.015)
1,1-Dimethylethanol	0.003 (0.001)	—	0.024 (0.008)	—
1,1,1-Trichloroethane	0.097 (0.018)	—	0.140 (0.026)	0.070 (0.013)
Methanol	—	0.010 (0.008)	0.014 (0.011)	0.020 (0.015)
2-Propanol	0.322 (0.131)	0.002 (0.001)	0.032 (0.013)	0.132 (0.054)
Dichloromethane	0.013 (0.004)	0.021 (0.006)	0.036 (0.010)	0.069 (0.020)
Ethanol	0.089 (0.048)	0.102 (0.054)	0.034 (0.018)	0.194 (0.103)
Benzene	0.005 (0.002)	0.001 (<0.001)	0.005 (0.001)	0.003 (0.001)
Hexamethylcyclotrisiloxane	0.111 (0.012)	0.018 (0.002)	0.017 (0.002)	0.027 (0.003)
n-Pentanal	0.057 (0.016)	0.070 (0.020)	0.036 (0.010)	0.063 (0.018)
4-Methyl-2-pentanone	—	—	0.010 (0.002)	0.008 (0.002)
Toluene	0.049 (0.013)	0.011 (0.003)	0.049 (0.013)	0.060 (0.016)
C_{10}-alkane	—	—	0.034 (0.006)	0.012 (0.002)
Acetic acid, n-butyl ester	0.016 (0.004)	—	0.004 (0.001)	0.005 (0,001)
n-Hexanal	0.031 (0.008)	0.045 (0.011)	0.029 (0.007)	0.020 (0.005)
C_{11}-alkane	—	—	0.009 (0.001)	0.012 (0.001)
C_{11}-alkane	—	—	0.031 (0.005)	—
Ethyl benzene	0.006 (0.002)	0.004 (0.001)	0.002 (<0.001)	0.002 (<0.001)
1-Butanol	0.009 (0.003)	0.003 (0.001)	0.006 (0.002)	—
C_{11}-alkane	—	—	0.024 (0.004)	—
C_{11}-alkane	—	—	0.032 (0.005)	—
1,4-Dimethylbenzene	0.003 (0.001)	0.001 (<0.001)	0.006 (0.001)	0.02 (<0.001)
1,3-Dimethylbenzene	0.018 (0.004)	0.009 (0.002)	0.021 (0.005)	0.009 (0.002)
C_{12}-alkane	—	—	0.031 (0.005)	—
C_{12}-alkane	—	—	0.022 (0.004)	—
3-Heptanone	0.037 (0.008)	—	0.067 (0.015)	0.042 (0.009)
C_{12}-alkane	—	—	—	0.032 (0.005)
1,2-Dimethylbenzene	0.020 (0.004)	0.004 (0.001)	0.015 (0.003)	0.017 (0.004)
2-Heptanone	0.021 (0.004)	—	0.052 (0.011)	0.016 (0.004)
Heptanal	—	—	—	0.042 (0.009)
n-Propylbenzene	0.008 (0.002)	—	0.014 (0.003)	0.010 (0.002)
C_3-substituted benzene	0.004 (0.001)	—	0.008 (0.001)	0.004 (<0.001)
Acetic acid, 2-ethoxy-ethylester	0.008 (0.002)	0.005 (0.001)	0.008 (0.001)	0.011 (0.002)
C_3-substituted benzene	—	—	0.005 (0.001)	0.003 (<0.001)
C_3-substituted benzene	—	—	0.002 (<0.001)	—
C_3-substituted benzene	0.014 (0.003)	—	0.026 (0.006)	0.015 (0.003)
C_3-substituted benzene	—	—	—	0.002 (<0.001)
C_4-substituted benzene	0.005 (0.001)	—	0.005 (0.001)	0.005 (0.0001)
C_3-substituted benzene	—	—	0.010 (0.002)	—
n-Butylbenzene	0.003 (<0.001)	—	0.005 (<0.001)	0.002 (<0.001)
C_4-substituted benzene	—	—	0.002 (<0.001)	—
C_4-substituted benzene	—	—	0.002 (<0.001)	0.003 (<0.001)
C_4-substituted benzene	—	—	0.006 (0.001)	
C_4-substituted benzene	—	—	0.002 (<0.001)	0.001 (<0.001)
C_4-substituted benzene	—	—	0.002 (0.001)	—

Note: Here and in Tables 3, 4 and 7, concentrations are given in mg/m^3, values in parentheses are parts per million.

Table 3. Analysis of MTKK-2 atmosphere

Compound	Cylinder		
	1	3	4
n-Pentane	—	—	0.215 (0.073)
Ethanal	0.346 (0.192)	4.724 (2.626)	1.708 (0.949)
Trichlorofluoromethane	0.020 (0.004)	—	0.160 (0.028)
1,1,2-Trichloro-1,2,2-trifluoro-ethane	0.046 (0.006)	13.091 (1.707)	7.183 (0.937)
2-Methyl pentane	—	2.625 (0.746)	0.277 (0.077)
n-Hexane	—	1.362 (0.387)	0.113 (0.032)
1,1-Dichloroethane	—	0.011 (0.003)	0.019 (0.005)
Carbon disulfide	—	0.002 (<0.001)	0.001 (<0.001)
Methyl cyclopentane	—	1.901 (0.553)	0.320 (0.093)
Propanal	0.140 (0.056)	0.539 (0.227)	0.587 (0.248)
2-Propanone	0.408 (0.172)	0.982 (0.414)	1.017 (0.429)
C_7-aliphatic hydrocarbon	—	0.144 (0.035)	—
n-Heptane	—	0.446 (0.109)	0.178 (0.043)
Cyclohexane	0.006 (0.002)	0.082 (0.018)	—
C_8-aliphatic hydrocarbon	—	0.082 (0.018)	0.091 (0.020)
C_8-aliphatic hydrocarbon	—	0.202 (0.043)	0.066 (0.014)
C_8-olefinic hydrocarbon	—	0.050 (0.011)	0.147 (0.032)
C_8-olefinic hydrocarbon	—	0.247 (0.053)	0.150 (0.032)
Methanol	—	0.534 (0.408)	0.407 (0.311)
n-Butanal	0.108 (0.036)	0437 (0.148)	0.464 (0.157)
C_8-olefinic hydrocarbon	—	0.093 (0.020)	0.050 (0.011)
2-Butanone	0.047 (0.016)	0.295 (0.100)	0.376 (0.127)
C_8-aliphatic hydrocarbon	—	0.207 (0.044)	0.640 (0.137)
2-Methyl-2-propanol	—	2.247 (0.742)	0.368 (0.122)
C_8-aliphatic hydrocarbon	—	0.904 (0.194)	0.426 (0.092)
1,1,1-Trichloroethane	—	0.262 (0.048)	0.284 (0.052)
C_8-aliphatic hydrocarbon	—	0.819 (0.176)	—
Ethanol	1.114 (0.592)	2.065 (1.098)	2.223 (1.182)
Dichloromethane	0.495 (0.142)	—	—
n-Octane	—	0.645 (0.138)	0.239 (0.051)
Benzene	—	0.089 (0.028)	0.019 (0.006)
C_9-aliphatic hydrocarbon	—	0.168 (0.032)	0.068 (0.013)
C_9-aliphatic hydrocarbon	—	0.143 (0.027)	0.114 ,0.022)
C_9-aliphatic hydrocarbon	—	0.237 (0.045)	0.102 (0.020)
C_9-aliphatic hydrocarbon	—	0.091 (0.017)	0.185 (0.035)
C_9-aliphatic hydrocarbon	—	0.076 (0.014)	0.058 (0.011)
C_9-aliphatic hydrocarbon	—	0.211 (0.040)	0.115 (0.022)
Trichloroethane	—	—	0.012 (0.002)
2-Methyl-1-propanol	0.034 (0.011)	—	—
n-Pentanal	—	—	0.510 (0.145)
C_9-olefinic hydrocarbon	—	—	0.197 (0.038)
C_9-olefinic hydrocarbon	—	0.188 (0.036)	—
C_9-aliphatic hydrocarbon	—	0.876 (0.167)	0.592 (0.113)
C_9-aliphatic hydrocarbon	—	0.746 (0.143)	0.596 (0.114)
C_9-aliphatic hydrocarbon	—	0.069 (0.013)	0.092 (0.018)
n-Nonane	0.018 (0.003)	0.368 (0.070)	0.342 (0.065)
C_{10}-aliphatic hydrocarbon	—	—	0.158 (0.030)
Trimethyl silanol	—	—	0.098 (0.027)
Toluene	0.335 (0.089)	63.877 (16.980)	20.352 (5.410)
C_{10}-aliphatic hydrocarbon	—	0.329 (0.057)	0.767 (0.132)
C_{10}-aliphatic hydrocarbon	—	0.244 (0.042)	0.179 (0.031)

Table 3. (continued)

Compound	Cylinder		
	1	3	4
C_{10}-aliphatic hydrocarbon	—	0.234 (0.041)	0.410 (0.070)
C_{10}-aliphatic hydrocarbon	0.025 (0.004)	0.191 (0.033)	0.318 (0.055)
C_{10}-aliphatic hydrocarbon	—	0.292 (0.050)	0.291 (0.050)
C_{10}-aliphatic hydrocarbon	—	—	0.135 (0.023)
C_{10}-aliphatic hydrocarbon	—	—	0.129 (0.022)
n-Decane	—	—	0.067 (0.011)
Ethyl benzene	0.003 (0.001)	0.409 (0.094)	0.256 (0.059)
C_{11}-aliphatic hydrocarbon	—	—	0.706 (0.122)
C_{11}-aliphatic hydrocarbon	—	—	0.250 (0.034)
1,4-Dimethylbenzene	0.004 (0.001)	—	0.327 (0.075)
C_{11}-aliphatic hydrocarbon	0.021 (0.004)	—	0.489 (0.077)
n-Butanol	0.015 (0.005)	0.055 (0.018)	0.086 (0.029)
1,3-Dimethylbenzene	0.022 (0.005)	1.383 (0.319)	1.182 (0.272)
C_{11}-aliphatic hydrocarbon	—	—	0.063 (0.010)
C_{11}-aliphatic hydrocarbon	0.044 (0.007)		0.643 (0.101)
C_{11}-aliphatic hydrocarbon	—	0.326 (0.051)	0.301 (0.047)
1,2-Dimethylbenzene	0.020 (0.005)	0.822 (0.190)	0.893 (0.206)
C_{11}-aliphatic hydrocarbon	—	—	0.232 (0.033)
C_{11}-aliphatic hydrocarbon	—	0.187 (0.029)	—
2-Ethylhexanal	—	—	0.428 (0.092)
n-Propylbenzene	0.002 (<0.001)	0.108 (0.022)	0.155 (0.032)
C_3-substituted benzene	0.010 (0.002)	0.355 (0.072)	0.548 (0.112)
6-Methyl-2-heptanone	—	—	0.066 (0.013)
C_3-substituted benzene	0.002 (<0.001)	0.118 (0.024)	0.187 (0.038)
C_3-substituted benzene	0.002 (<0.001)	0.073 (0.15)	0.137 (0.028)
C_3-substituted benzene	0.002 (<0.001)	0.313 (0.064)	0.561 (0114)
C_{12}-aliphatic hydrocarbon	0.013 (0.002)	—	0.044 (0.006)
C_4-substituted benzene	0.002 (<0.001)	0.015 (0.003)	0.029 (0.005)
C_4-substituted benzene	—	0.053 (0.010)	0.120 (0.022)
C_4-substituted benzene	0.001 (<0.001)	0.047 (0.009)	0.147 (0.027)
C_3-substituted benzene	—	0.079 (0.016)	—
C_4-substituted benzene	—	0.038 (0.007)	0.086 (0.016)
C_4-substituted benzene	0.003 (<0.001)	—	0.044 (0.008)
Indan	—	0.009 (0.002)	0.021 (0.004)
C_4-substituted benzene	—	0.019 (0.003)	0.042 (0.008)
C_4-substituted benzene	—	0.019 (0.003)	0.042 (0.008)
C_4-substituted benzene	—	0.017 (0.002)	0.082 (0.015)
C_4-substituted benzene	—	0.036 (0.007)	9.010 (0.002)
C_4-substituted benzene	—	—	0.029 (0.005)
C_{13}-aliphatic hydrocarbon	0.013 (0.002)	—	—
Benzaldehyde	—	0.016 (0.004)	0.016 (0.004)
C_4-substituted benzene	0.002 (< 0.001)	0.005 (< 0.001)	0.029 (0.005)
C_4-substituted benzene	0.001 (< 0.001)	0.005 (< 0.001)	0.014 (0.002)
C_4-substituted benzene	0.003 (< 0.001)	0.014 (0.002)	0.033 (0.006)
C_{14}-aliphatic hydrocarbon	0.058 (0.008)	0.252 (0.034)	0.439 (0.054)
Naphthalene	—	—	0.011 (0.002)
Carbon monoxide	0.572 (< 0.500)	0.572 (< 0.500)	0.572 (<0.500)
Methane	4.063 (7.127)	2.415 (3.691)	3.805 (5.816)

Table 4. Analysis of MTKK-3 atmosphere

Compound	Cylinder			
	1	2	3	4
Carbon monoxide	—	2.610 (2.28)	0.80 (0.07)	1.076 (0.94)
Methane	1.662 (2.54)	4.933 (7.54)	1.995 (3.05)	2.970 (4.54)
Pentane	0.015 (0.005)	0.009 (0.003)	0.003 (0.001)	—
Bromotrifluoromethane	0.225 (0.037)	1.816 (0.298)	10.577 (1.736)	16.292 (2.674)
Trichlorofluoromethane	0.007 (0.001)	0.006 (0.001)	0.011 (0.002)	0.062 (0.011)
1,1,2-Trichloro-1,2,2-trifluoroethane	0.238 (0.031)	0.215 (0.028)	0.836 (0.109)	2.124 (0.277)
Ethanal	0.079 (0.044)	0.210 (0.117)	0.041 (0.023)	0.133 (0.074)
Propanal	0.009 (0.004)	0.012 (0.005)	0.014 (0.006)	0.040 (0.017)
2-Propanone	0.064 (0.027)	0.202 (0.085)	0.028 (0.012)	0.100 (0.042)
Heptane	—	—	0.004 (0.001)	—
1-Butanal	0.012 (0.004)	0.012 (0.004)	0.012 (0.004)	0.035 (0.012)
2-Butanone	0.018 (0.006)	0.015 (0.005)	0.035 (0.012)	0.065 (0.022)
2-Methyl-2-propanol	0.006 (0.002)	0.006 (0.002)	0.006 (0.002)	0.003 (0.001)
1,1,1-Trichloroethane	0.005 (0.001)	0.005 (0.001)	0.022 (0.004)	0.060 (0.011)
2-Propanol	0.132 (0.054)	0.069 (0.028)	0.132 (0.070)	0.037 (0.015)
Ethanol	0.269 (0.143)	2.272 (1.208)	0.208 (0.085)	0.248 (0.132)
Dichloromethane	0.038 (0.011)	0.087 (0.025)	0.035 (0.010)	0.104 (0.030)
Benzene	<0.003 (<0.001)	<0.003 (<0.001)	0.003 (0.001)	0.006 (0.002)
Hexamethylcyclotrisiloxane	0.009 (0.001)	0.009 (0.001)	—	<0.009 (<0.001)
Methylethylcyclopentane	—	—	0.009 (0.002)	0.014 (0.003)
n-Pentanal	0.035 (0.010)	0.021 (0.006)	0.021 (0.006)	—
C_8-alkane	—	—	0.009 (0.002)	0.009 (0.002)
4-Methyl-2-pentanone	<0.004 (<0.001)	—	—	0.020 (0.050)
Trichloroethane	—	—	0.016 (0.003)	0.011 (0.002)
Toluene	0.022 (0.006)	0.011 (0.003)	0.083 (0.022)	0.158 (0.042)
C_9-alkane	—	—	—	0.010 (0.002)
C_9-alkane	<0.005 (<0.001)	0.005 (0.001)	0.016 (0.002)	0.030 (0.006)
C_9-alkane	—	—	—	0.045 (0.009)
C_9-alkane	<0.005 (<0.001)	0.005 (0.001)	0.002 (0.010)	0.020 (0.004)
Ethylbenzene	<0.004 (<0.001)	<0.004 (0.001)	<0.004 (<0.001)	0.004 (0.001)
1,4-dimethylbenzene	<0.004 (0.001)	—	<0.004 (<0.001)	0.004 (0.001)
1,3-dimethylbenzene	0.004 (0.001)	0.004 (0.001)	0.004 (0.001)	0.009 (0.002)
1,2-dimethylbenzene	0.004 (0.001)	<0.004 (<0.001)	0.004 (0.001)	0.009 (0.002)
C_{10}-alkane	0.006 (0.001)	0.006 (0.001)	0.023 (0.004)	0.029 (0.005)
C_{10}-alkane	0.006 (0.001)	0.012(0.002)	0.006 (0.001)	0.035 (0.006)
C_{10}-alkane	—	—	0.006 (0.001)	0.035 (0.006)
C_{10}-alkane	0.006 (0.001)	0.006 (0.001)	0.017 (0.003)	—
C_{10}-alkane	0.006 (0.001)	0.006 (0.001)	0.017 (0.003)	0.046(0.008)
C_{11}-alkane	—	—	0.013 (0.002)	—
C_{12}-alkane	—	—	0.007 (0.001)	

There were 4 cylinders aboard MTKK-1, MTKK-2 and MTKK-3 to collect samples by the whole-gas sampling method. In view of weight restrictions, there was only 1 cylinder for whole-gas sampling aboard MTKK-4 and MTKK-5. Due to technical difficulties, the device for absorbed-gas sampling was used only in the missions aboard MTKK-1 and MTKK-2. Cylinder No 2, which was intended for whole-gas sampling was not used inflight to collect samples.

Due to the high level of toluene found during the flight of MTKK-2, preflight samples were taken during ground-based trials before the mission of MTKK-3. The results of these analyses are listed in Table 7.

Table 5.
Analysis of MTKK-4 atmosphere

Compound	Cylinder No 1
Methane	135,540 (89,351)
Dichlorodifluoromethane	0,033 (0,162)
Bromotrifluoromethane	0,383 (2,318)
1,1,2-trichloro-1,2,2-trifluoroethane	1,332 (10,214)
2-Propanone	0,012 (0,028)
Ethanol	0,409 (0,769)

Note: Here and in Table 6, concentrations are listed in parts per million; in parentheses they are given in mg/m^3.

Table 6.
Analysis of MTKK-5 atmosphere

Compound	Cylinder No 1
Methane	114,774 (75,108)
Carbon monoxide	1,021 (1,837)
Butene	0,683 (1,563)
1,1,2-trichloro-1,2,2-trifluoroethane	0,009 (0,069)
Ethanal	0,016 (0,029
2-Propanone	0,026 (0,061)
2-Butanone	0,003 (0,010)
Dichloromethane	0,006 (0,021)
2-Propanol	0,004 (0,009)
Ethanol	0,051 (0,096)
Benzene	0,001 (0,001)
1,4-Dimethylbenzene	0,001 (0,002)
1,3-Dimethylbenzene	0,001 (0,001)
Tolune	0,002 (0,008)
Siloxane	0,011 (0,174)
Siloxane	0,004 (0,072)
Siloxane	0,002 (0,040)
Total for all compounds	116,615 (79,101)

According to the data listed in Table 1, we see that there was a maximum quantity of compounds (99) in the crew cabin atmosphere during the MTKK-2 mission. There were 56 compounds in the crew cabin atmosphere during the MTKK-1 flight, 40 during the MTKK-3 flight, 17 in the MTKK-5 mission and 6 in the MTKK-4.

The concentrations of trace contaminant gases in the MTKK cabin atmosphere ranged from 28 ppm [parts per million] for methane to 0.001 ppm for 1,4-dimethylbenzene during the MTKK-1 flight. The concentrations of toxic gases in the atmosphere of MTKK-2 ranged from 17 ppm for toluene to less than 0.001 ppm for carbon disulfide. The concentrations of trace contaminant gases aboard MTKK-3 varied from 7.5 ppm in the case of methane to less than 0.001 ppm for benzene. This range for MTKK-4 was from 135 ppm (methane) to 0.033 ppm for freon-12. During the MTKK-5 mission, the concentration of methane was 115 ppm and benzene was less than 0.001 ppm.

Table 1 shows that 59 distinctly demonstrable compounds were identified during the 5 MTKK flights. In addition, on the basis of examining the results of these five missions, other compounds were detected (98), but they were identified only on the basis of functional grouping, for example, aliphatic hydrocarbons, alkanes, olefinic hydrocarbons, siloxanes and substituted benzenes.

The only problem related to trace concentrations of contaminant gases arose during the MTKK-2 mission after air samples from the MTKK-2 cabin were submitted to chemical analysis and the results were evaluated on the basis of the above-described equation. Since the concentration of toluene reached a level of 17 ppm, while maximum allowable concentration for a spacecraft is 20 ppm, the presence of several other compounds of the same toxicological group yielded a total (calculated using the equation) that exceed 1.

Table 7. Preflight analysis of MTKK-3 cabin atmosphere

Compound	Cylinder	
	1	2
Trichlorofluoromethane	0,939 (0,167)	1,709 (0,304)
1,1,2-Trichloro-1,2,2-trifluoroethane	0,284 (0,037)	1,020 (0,133)
Ethanal		0,189 (0,105)
Propanal		0,014 (0,006)
2-Propanone	<0,002 (<0,001)	0,007 (0,003)
Butanal		0,026 (0,009)
2-Butanone		0,006 (0,002)
1,1,1-Trichloroethane	0,005 (0,001)	0,016 (0,003)
Dichloromethane	0,031 (0,009)	0,059 (0,017)
2-Propanol		0,007 (0,002)
Benzene	<0,003 (<0,001)	<0,003 (<0,001)
Toluene		<0,004 (<0,001)
Carbon monoxide	<0,057 (0,05)	0,057 (<0,05)
Methane	1,091 (1,668)	1,067 (1,631)

Steps were taken immediately to correct this problem. The quantity of toluene used in the zone of the MTKK crew cabin was reduced. Before the MTKK-3 mission, we took and analyzed air samples from the Shuttle cabin. The results of this analysis are listed in Table 7. Table 7 shows that, with proper control, solvent toluene concentration was less than 0.004 ppm. More rigid control was established on the basis of this experiment, with regard to determination of types and concentrations of solvents, paints and other substances that are permitted for use between missions in the crew cabin zone.

Conclusion

A distinct toxicological program was formulated and adopted for support of the Space Shuttle Program. Toxicological support of Shuttle spaceflights is very important, since cabin air presents the greatest potential toxicological hazard for MTKK crew members. The most harmful concentrations of toxic compounds outgassed in the crew cabin atmosphere were discovered during evaluation of constructions materials selected for MTKK. However, there was an instance during a mission when an inadmissibly high concentration of toluene vapor and other analogous compounds was formed in the cabin atmosphere.

With the exception of this incident, the MTKK cabin atmsophere was relatively clean and safe for vital functions of crews during spaceflights.

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